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# Characterization of Iron-Bearing Solids Used by Naturally-Occurring Microbes in the Anaerobic Degradation of Hydrocarbons

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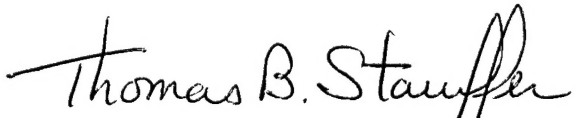

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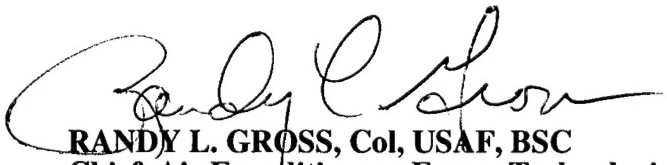

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**13. ABSTRACT** *(Maximum 200 words)*

This study investigated the availability of iron-bearing minerals for use by naturally-occurring, iron-reducing microbes in the anaerobic oxidation of hydrocarbon groundwater contaminants during a field experiment at Columbus Air Force Base, Columbus, Mississippi. While this research could find no statistically significant changes in the iron oxides in the aquifer materinal over time, this work did produce three important findings. First, the natural consortium of bacteria is shown to degrade hydrocarbons in a reducing environment in the presence of iron oxides. Second, there appears to be some consumption of the iron oxides during natural attenuation near the source emplacement. Third, formation of magnetite cannot be used as a primary indicator of microbial degradation in the field, since laboratory studies showed that magnetite was produced by both biotic and abiotic systems.

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Dedication

This thesis is dedicated in loving memory of my grandparents, Otis P. and Amy H. Thomaston.

# 1. Table of Contents

## 2. Acknowledgments

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## 3. List of Tables

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## 5. Abstract

This study investigated the availability of iron-bearing minerals for use by naturally-occurring, iron-reducing microbes in the anaerobic oxidation of hydrocarbon groundwater contaminants during a field experiment at Columbus Air Force Base (CAFB), Columbus, Mississippi. It includes the characterization of the aquifer materials in site samples, and of solids from laboratory experiments designed to identify iron mineral microbial reactions that may occur in contaminated aquifers. This work is a component of the Natural Attenuation Study (NATS) field experiment, conducted by the USAF to determine the viability of natural attenuation as an effective remediation technique for organic contaminants in aquifers.

Several instrumental and chemical analysis techniques were used to study the bulk mineralogy and iron phases present in the CAFB aquifer material and in laboratory solids from laboratory experiments. Powder x-ray diffraction was used to determine the minerals present in the aquifer, and for the identification of amorphous iron phases and identify secondary iron phases formed in the laboratory studies. Ferrous and ferric iron were extracted from solid phases with HCl extractions. Total extracted iron was analyzed by inductively coupled plasma, atomic emission spectrophotometry, and by flame atomic absorption. Ferrous iron concentration was determined by spectrophotometric analysis of its complex with ferrozine. In addition, scanning electron microscopy coupled with energy dispersive x-ray analyses was used to obtain information on average iron content of mineral coatings. The scanned images also provided topological information about the sediment and secondary minerals. Specific surface area was determined by nitrogen gas absorption.

While this research could find no statistically significant changes in the iron oxides in the aquifer material over time. This work produced three important findings. First, the natural consortium of bacteria is shown to degrade hydrocarbons (HC) in a reducing environment in the presence of iron oxides. Second, there appears to be some consumption of the iron oxides during NATS near the source emplacement. Third, formation of magnetite can not be used as a primary indicator of microbial degradation in the field, since laboratory studies showed that magnetite was produced both biotic and abiotic systems. There are also several suggestions given for further research including using larger hydrocarbon sources and better statistical approaches to determine the heterogeneity of alluvial aquifers.

# CHARACTERIZATION OF IRON-BEARING PHASES USED BY NATURALLY-OCCURRING MICROBES IN THE ANAEROBIC DEGRADATION OF HYDROCARBONS

## 6. Introduction and Background:

This work investigated the role of iron bearing minerals in natural attenuation of organic contaminants in aquifers. Natural attenuation is a term apparently first used by Barker et al. (1987) to describe the set of physical and chemical processes occurring in aquifers that diminish contaminant concentrations over time without human intervention. Natural attenuation has been proposed as a technique for remediation of hydrocarbons in contaminated aquifers. For remediation by natural attenuation to be considered feasible, biodegradation must be shown to occur, and the maximum extent of the dissolved contaminant plume migration must be estimated (Borden et al., 1997). A natural attenuation study (NATS), directed and supported by the United States Air Force (USAF) Armstrong Laboratory, Environmental Quality Laboratory (AL/EQL), was designed to examine the effects of naturally-occurring processes, including microbial degradation, dilution, and evaporation, on the transport and fate of an organic contaminant plume. As the goal of remediation is to reduce contaminant concentration in an aquifer to a level that no longer threatens human health or the environment (Nyer, 1997), natural attenuation may be the best choice remediation design at sites where other remediation techniques may cause significant environmental damage (Boggs et al., 1993).

Uncontrolled release of hydrocarbons to subsurface systems is widespread. Leaking underground storage tanks, improper disposal of hazardous materials in landfills and excessive use of agricultural chemicals are

all important sources of contamination of groundwater (Mihelcic et al., 1995). NATS focuses on contamination by petroleum derived fuels, especially the primary dissolved components known as BTEX (benzene, toluene, ethylbenzene and xylene). BTEX makes up less than 20 percent of fuel mixtures like jet fuel, gasoline and diesel fuel, but accounts for 82 percent and 98 percent of the dissolved compounds from fresh JP-4 and gasoline, respectively (Wiedemeier, et al., 1996).

Contaminant plume movement in an aquifer is caused by advection and dispersion of the water-born contaminants. Factors controlling the attenuation of groundwater contaminants include dilution, retardation, precipitation, ion exchange, and abiotic and microbial degradation (Nyer, 1997, Wiedemeier et al., 1996). Of these, only the degradation processes (e.g.. oxidation) transform the hydrocarbons to innocuous products, and microbial degradation is generally much faster than abiotic.

Upon introduction of hydrocarbons into an aquifer, zones of oxidation of the contaminant plume form by sequential consumption of available oxidizing agents (e.g. $O_2$, $NO_3^-$, and Fe (III)). An idealized distribution of terminal oxidation processes in a plume spreading from a source of hydrocarbon contaminants includes, in order of increasing redox potential and distance from the source: methanogenic, sulfate-reducing, Fe (III)-reducing, nitrate and Mn (IV)-reducing, and oxic processes (Lovley et al., 1994). The existence of each of these zones requires availability of the associated electron acceptor within the aquifer.

Common evidence of natural attenuation of hydrocarbons at a field site by microbial processes includes: documented loss of contaminants; chemical data showing increased concentration of intermediates and metabolites, and concomitant decreased concentration of terminal electron acceptors in the aquifer; laboratory culture studies, showing microbial degradation (Wiedemeier et al., 1996; Wilson and Madsen, 1996). In addition, several other geochemical parameters provide evidence for bioremediation. Differences between the $\delta^{13}C$ values of hydrocarbons and of indigenous carbon sources (e.g. plant matter, soil carbonates) can be used to trace the origins of metabolic end products (Conrad et al., 1997). Also, the concentration of dissolved $H_2$ has been shown to correspond to redox potential, and thus can identify which oxidation process predominates at specific locations in an aquifer (Lovley et al., 1994). Finally, in situ molecular biology measurements using gene probes have shown directly the activity of indigenous microorganisms in oxidation of the source hydrocarbons during the NATS experiment (Stapleton et al., 2000).

The observed distribution of microbial activity in an aquifer depends, in part, on the availability of nutrients that, in turn, is controlled primarily by the physical and chemical heterogeneity. Low conductivity inclusions in an aquifer may greatly enhance microbial degradation of hydrocarbons due to prolonged availability of substrate (Murphy et, al., 1997).

Iron-reducing bacteria that completely oxidize hydrocarbons have recently been discovered. *Geobacter metallireducens was* the first microorganism found to completely, anaerobically oxidize aromatic hydrocarbons to $CO_2$ using Fe (III)

as a terminal electron receptor (Lovley and Phillips, 1988). It also was the first pure culture capable of anaerobically oxidizing toluene, a common component of petroleum products (Lovley and Phillips, 1988; Lovley and Lonergan, 1990). Other bacteria that use Fe (III) to completely oxidize hydrocarbons have since been cultured from riverine sources. For example, *Shewanella alga* strain BrY grows in a medium with synthetic goethite as the only oxidant, reducing the Fe (III) by 8-18% (Roden and Zachara, 1996) and *Pelobacter carbinolicus* is able to use both Fe (III) and $S^0$ as terminal oxidants (Lovley et al., 1995). This evidence of iron-reduction by bacteria in the laboratory suggests that their action may be an important component of natural attenuation of hydrocarbons in aquifers.

The Fe (III) minerals occurring in a contaminated aquifer may govern their contribution to natural attenuation. For example, some studies of aquatic sediments and submerged soil show that crystalline iron (III) oxides like goethite ($\alpha$-FeOOH) and hematite ($\alpha$-$Fe_2O_3$) may not be reducible by microorganisms, but amorphous and poorly crystalline iron (III) oxides are readily reduced (Phillips et al., 1993; Lovley and Phillips, 1988). Other studies have shown that Fe (III)-reducing bacteria may produce significant quantities of Fe (II) in anaerobic soils and subsurface environments where crystalline iron (III) oxides such as goethite are the dominant forms of Fe (III) (Roden and Zachara, 1996). Differences in mineral utilization may be a function of the naturally-occurring consortium of bacteria. Reduction of iron (III) oxides can produce significant geochemical changes in an aquifer. Reduction releases soluble Fe (II) and other adsorbed species (e.g. phosphates and trace metals), and provides a reactive iron oxide

surface capable of participating in secondary redox or mineral-forming reactions (Roden and Zachara, 1996). In any case, these considerations indicate that structural and compositional analysis of Fe (III) minerals are required for investigations of natural attenuation of hydrocarbons in aquifers.

This thesis work was designed to investigate changes in the iron-bearing minerals in the Columbus AFB aquifer associated with hydrocarbon degradation in the NATS experiment. The site aquifer has low concentrations of dissolved sulfate and nitrate and little solid manganese oxides, so microbially-mediated iron (III) reduction of the emplaced hydrocarbons is expected to be the primary oxidant once the oxygen has been depleted. Experiments reported here were designed to characterize changes in the Fe (III)-bearing phases due to microbial degradation and to assess the availability of these phases to support degradation processes.

### *6.1. Description of the NATS Project*

The Natural Attenuation Study (NATS) field experiment was initiated in November 1995 in an unconfined aquifer at Columbus AFB (Fig. 1). It was designed to demonstrate and quantify natural attenuation at a contamination site, and thus required definition of initial (source) conditions and the measurement of contaminant transport and fate during the evolution of the contaminant plume.

The shallow unconfined aquifer at the NATS site is an alluvial terrace deposit averaging 11 meters in thickness, composed of poorly sorted to well-sorted sandy gravel and gravely sand with minor amount of silt and clay. Marine sediment of the Eutaw Formation consisting of fine sand silt and clay forms an aquitard below this alluvial aquifer. A detailed site description of the site and its geology is given in Boggs et al. (1992).

The hydrology of the aquifer was determined in earlier studies (Boggs et al. 1993; MacIntyre et al. 1993). The local groundwater has low organic carbon, nitrogen, phosphorous and sulfate concentrations. Mean dissolved concentrations for sulfate, potassium and nitrate-nitrite are 2.0 mg/L, 0.6 mg/L and 1.5 mg/L, respectively (Boggs et al., 1993). The dissolved oxygen in the aquifer ranges from 2.6 to 3.8 mg/L (Macintyre, 1993). Iron oxide content of the aquifer material is high, ranging from 2.0 to 3.9 percent by weight for particle diameters less than 2mm, and from 0.5 to 0.8 percent for particles greater than 2mm (Boggs et al., 1993). Thus abundant iron oxides minerals are available for hydrocarbon oxidation. The aquifer sediment organic carbon concentrations are quite low, ranging from 0.02 to 0.06 percent (MacIntyre et al., 1991).

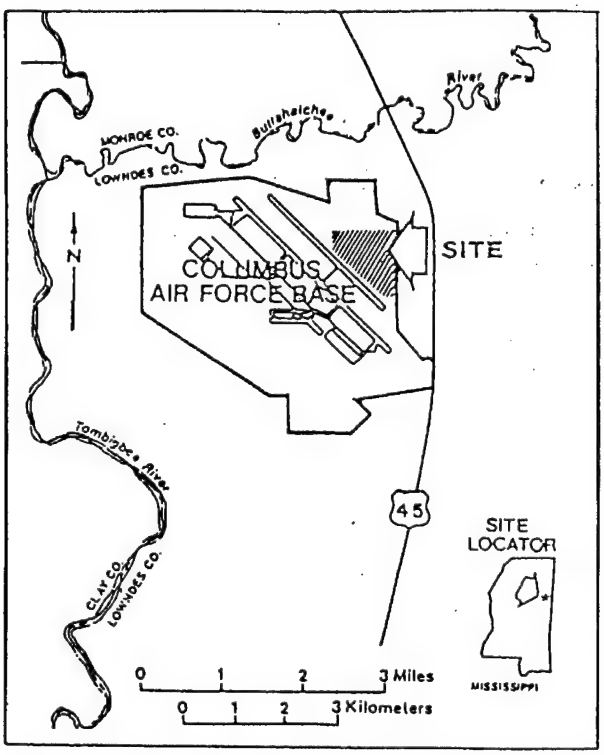
The NATS experiment was begun by emplacing a large hydrocarbon, non-aqueous phase liquid (NAPL) mass in the saturated zone of the aquifer (see details in section 6.1.2). Leaching, transport and geochemical fate of the NAPL components were then observed over space and time to measure hydrocarbon attenuation by processes including microbial degradation and dilution. Agencies cooperating in NATS were the USAF AL/EQC-OL, Tennessee Valley Authority,

College of William and Mary, University of Tennessee, and Florida State University. Each agency investigated a particular process/component of natural attenuation. The study reported here focuses on the role of iron minerals in hydrocarbon oxidation. The need for understanding of natural attenuation processes to support remediation actions is apparent from conclusions of the Groundwater Cleanup Alternatives Committee report (NAS, 1994). Based on subjective and qualitative analysis of past remediation operations in contaminated aquifers, the report concludes that existing active remediation actions are ineffective or economically feasible at most sites. Thus natural attenuation becomes an attractive alternative, presuming it can be proven effective.

A satisfactory site remediation by natural attenuation is indicated by maintenance of steady-state contaminant concentrations at the down-gradient edge of the plume, where concentrations must be below the applicable water quality standards for the site. Natural attenuation is considered successful if this condition is realized within the site land parcel.

In the NATS experimental demonstration of natural attenuation, initial conditions of source location, amount, composition and emplacement time, and of hydrological parameters such as hydraulic heads and conductivities were established. With this information, the experiment results can be used to show the attainment of a natural attenuation plume condition, and to address the feasibility of natural attenuation actions at other sites, as specified in the objectives (see Section 6.1.1).

**Figure 1: Location of NATS Site.**



### 6.1.1. Objectives of the NATS program

The primary objectives of NATS were to show the development, from known conditions, of a steady state hydrocarbon plume distribution maintained by natural attenuation, and to provide information on the chemical, geomicrobiological and transport processes that are components of this natural attenuation. Observations at existing contamination sites cannot provide this information on natural attenuation processes in aquifers, because initial conditions and histories are poorly known.

The NATS results provide a scientific framework for managerial decisions regarding implementation of natural attenuation at other aquifer contamination sites.

Several parameter measurement programs were included in NATS that contributed to achievement of these objectives. Concentrations of hydrocarbons, oxygen, ferrous iron, inorganic carbon, methane, and $^{13}C$ isotope abundances were measured in groundwater samples. Spatial and temporal distributions of these analytes were used to demonstrate the attenuation of the hydrocarbons leaching from the source region. Quantification of natural attenuation of the emplaced NAPL also required core sampling of aquifer materials in the source and downgradient regions to obtain information on changes in NAPL distribution, microbial abundances and activities, and aquifer mineral composition during the experiment. This was the first field experiment to

investigate natural attenuation of a large NAPL in an aquifer, and the initial conditions were chosen with the intent of attaining a steady state plume within the test site boundaries.

The NATS experimental design included the investigations reported here on the role of ferric iron oxide minerals in the oxidation (degradation) of hydrocarbons. Such information is needed to determine whether the aquifer has an oxidizing capacity in excess of that provided by the dissolved oxygen its groundwater. At sites where oxidation by iron oxides is significant, fate and transport prediction models for hydrocarbons in groundwater, such as BIOPLUME II (Rifai, 1989) that treat only oxidation by dissolved oxygen are inadequate. Since hydrocarbon oxidation by iron oxides has been found appreciable in NATS, the resulting NATS data set is now being used to develop a fate and transport model for hydrocarbon fuels that includes this oxidation (Widdowson, 2000). This model may then be applied to other site situations where iron oxides are present and dissolved oxygen is depleted. In order to support the evaluation of the contribution of iron oxides to hydrocarbon degradation by the activities of the iron bacteria (the subject of this thesis), aquifer material mineralogy, chemical composition, and oxidation capacities were determined.

### 6.1.2. Physical Description of NATS Experiment

The first stage of the NATS experiment was emplacement of the hydrocarbon NAPL mixture coated on native sand in a steel sheetpile lined source trench (Figure 2) from which water and sediment were removed. Excavation of 1040 $m^3$ of sediment and shoring of the sheetpile cell in the trench was done November 4-9, 1995. Mixing and placement of the hydrocarbon coated source material was done over seven hours beginning at noon November 10, 1995. Cement mixers were used to mix the sand fill with the hydrocarbon NAPL. Eight 3.75 $m^3$ batches were prepared and placed in the trench. Composition and amounts of hydrocarbons added, based on masses added to mixers, are given in Table 1. The source zone was cored within a month after the start of the experiment and analyzed for hydrocarbons, in order to prove that loss of hydrocarbons due to vaporization during emplacement was not significant.

A 5300L volume of bromide solution (128 mg/L KBr) was prepared on-site using local groundwater. Approximately one eighth of this solution was added to each of the eight sand-hydrocarbon mixer batches, and this bromide served as an inert (conservative) indicator of groundwater movement.

On completion of source placement, backfill was poured over the source material to a depth of about 0.5 m to minimize hydrocarbon vapor losses. The next morning the trench was backfilled to about 3 m below grade and dewatering terminated. Complete backfill of the trench and removal of shoring was done on

November 14, 1995, and sheetpiling was removed by pulling vertically on Nov 30- Dec 1, thereby commencing the NATS experiment by starting aquifer flow through the source zone.

**Figure 2: Hydrocarbon Source Zone**

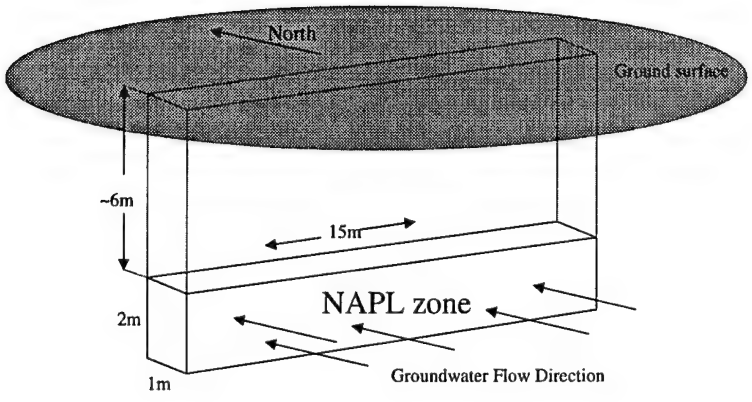


**Table 1: Composition of the hydrocarbon (NAPL) mixture emplaced in the source trench.**

| Compound | Mass (kg) | Mass Fraction of total | Mole Fraction of total | Solubility (mg/L) | Mixture Solubility (mg/L) |
|---|---|---|---|---|---|
| Decane | 856.42 | 0.7463 | 0.6878 | 0.009 | 0.006 |
| Benzene | 0.577 | 0.0005 | 0.0008 | 1760 | 1.6 |
| Toluene | 73.02 | 0.0636 | 0.0906 | 515 | 54 |
| Ethylbenzene | 71.84 | 0.0626 | 0.0773 | 152 | 14 |
| p-xylene | 72.65 | 0.0633 | 0.0782 | 198 | 18 |
| Naphthalene | 73.11 | 0.0637 | 0.0652 | 100 | 12 |

### *6.2. Summary of groundwater solute temporal and spatial distributions*

Initial analysis of geochemical changes during NATS was done using solute concentrations for all wells in each of seven zones that were defined by distance normal to the long axis of the source box (Figure 3). Each zone extends laterally 12.5 meters from of a line normal to the long axis of the source with origin at the source center. Each well shown in Figure 3 contained a multilevel sampler with 20 to 30 separate sampling points spaced 0.38 meters apart in the vertical direction, spanning the thickness of the aquifer. Details of multilevel sampler construction are given in Boggs et al. (1988). The monitoring wells (identified by numbers shown in Figure 3) in the zones are:

-10 to 0m, No. 57- 59;

0 to 5m, No. 10-14, 38, 43, 209, 295-298;

5 to 10m, No. 15-21, 44-46, 210-214, 299, 300, 302;

10 to 15m, No. 22-27, 49, 50, 303-305;

15 to 20m, No. 28-32, 52, 65-69;

20 to 25m, No. 73-79;

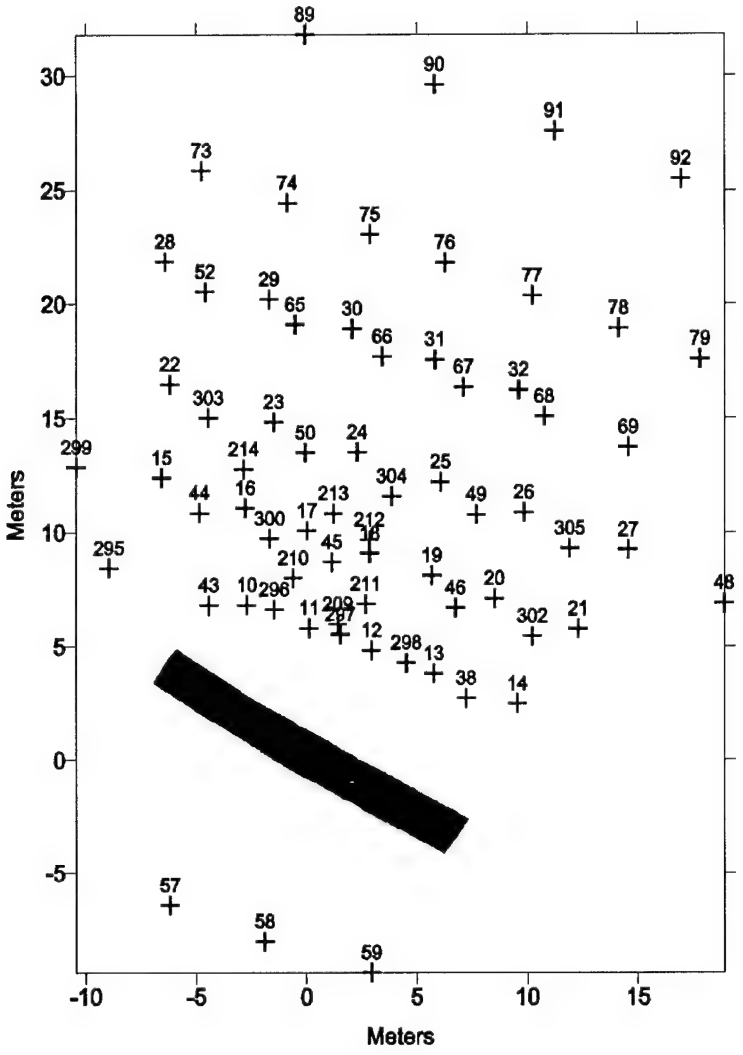
25 to 30m, No. 89-92.

Solute concentrations were averaged over all well point samples of all wells in each zone, with the process repeated for each sampling time ("snapshot"). These averages (shown in Appendix Table A1) provide a measure of spatial and temporal distributions that indicate geochemical changes occurring

in the experiment, including Fe (II) released by Fe (III) reduction during hydrocarbon oxidation. Limitations involved in use of these average concentrations as measures of geochemical change are that averaging obscures heterogeneity at lengths less than the selected zone size, and that well point spatial coverage within each zone may not allow determinations of "representative" or "true" values for the zones. Earlier experiments at the NATS site have shown heterogeneity at lengths less than 5m (MacIntyre et al, 1998), the length of the zones. Quantification of the effect of sample spatial density on the averages reported was not possible, as it would require installation of many more wells, which was precluded by cost and site disturbance considerations. Nonetheless, these zonal concentrations results can be used to examine changes in parameters that indicate of hydrocarbon degradation. Also correlation among the parameters can be used to identify biogeochemical processes occurring in natural attenuation of the source. Interpretation of the analytical results summarized in Appendix Table A1 is now being done on going by the NATS collaborators involved in collection of the aqueous chemistry data. However, an overview of the groundwater geochemical changes relevant to microbial use of iron oxides during the hydrocarbon degradation is provided by examination of Appendix Table A1. Primary among these considerations is the relative transport of the bromide and hydrocarbon (HC) pulses, and the appearance of ferrous iron and methane pulses. The bromide pulse can be followed spatially and temporally throughout the entire monitored aquifer extending out approximately 30 meters from the source, where the HC pulse

primarily extends only 10 m from the source with a trivial amount shown to have extended 15 meters. The methane and Fe (II) pulses were maximized in the 0-5 meter zone from the source. These results clearly show that the hydrocarbons are being degraded and that conditions are at least reducing enough for iron oxide reduction to have taken place. Therefore, changes in iron mineralogy may be noticeable up to 10 meters from the source material.

**Figure 3: Position of Wells Forming Zones Near the NAPL Source.**



## *6.3. Overview of this research on the role of iron oxides in the oxidation of hydrocarbons.*

### 6.3.1. Hypotheses

In order to determine if naturally occurring microbes can use iron oxides associated with aquifer materials, several questions must be answered that relate to the following general hypothesis.

General Hypothesis*: Microbial use of Fe (III) minerals for oxidation of hydrocarbons will produce changes in the iron minerals of NATS site sediments.*

Groundwater analysis results from the NATS collaborators provide strong evidence that microbes used Fe (III) minerals, namely the appearance of dissolved Fe (II) as well as the appearance of breakdown products of the organic contaminants. Changes in the aquifer material due to dissimilatory Fe (III) reduction by microbes may be manifest in several ways, as outlined in the following secondary hypotheses:

H1: *Total iron content of the near source sediments decreases with time.*

Justification: Reduction of Fe (III) solids to Fe (II) results in the solubilization of the Fe (II), and thus releases iron from the sediments to the moving groundwater.

TEST: Total iron in the sediments and Fe/Si from EDS analysis should show a temporal decrease. Also, there should be a loss in the XRD peaks attributed to Fe (III) phases.

H2: *Fe (II) released during Fe (III) reduction is re-deposited in downfield sediments, showing that Fe (II) is non-conservative.*

Justification: Aqueous chemistry results show that Fe (II) released by processes in the source region does not travel with the bromide tracer.

TEST: Identification of re-deposited Fe (II), probably as sorbed Fe (II) or Fe (II)-containing minerals, detectable by an increase over time in amorphous iron at downfield locations based on amorphous iron extractions of the core samples.

H3: *Amorphous Fe (III) will be preferentially consumed during microbial degradation of organic contaminants.*

Justification: Previous research has demonstrated the availability of amorphous Fe (III) phases for microbial use and the greater difficulty in utilizing more crystalline forms of Fe (III) oxides (Phillips et al., 1993; Lovley and Phillips, 1988; Roden and Zachara, 1996). This preferential utilization of amorphous iron should happen in the NATS experiment, but may be undetectable due to the limited amount of hydrocarbon added. However, the change may be detectable in laboratory experiments.

TEST: Laboratory microbial experiments using aquifer material will analyze amorphous Fe (III) and total Fe, documenting changes in the ratio of amorphous to total Fe.

The general hypothesis and the first two secondary hypotheses relate to results of the field experiment, and the first and third hypotheses relate to the laboratory microbial experiments described below.

Another, somewhat separate, laboratory experiment (the magnetite experiment) was conducted to explore the character of the secondary Fe (II) phases formed during oxidation of organics and to test the following hypothesis.

*Hypothesis: Secondary Fe (II)-bearing phases, in particular magnetite, form only by microbial processes, and do not form in abiotic systems.*

Justification: Magnetite has been found in contaminated aquifers under reducing conditions and is considered a by-product of microbial iron reduction. If magnetite forms only in the presence of iron-reducing microbes, its production could be a useful indicator of microbial degradation of hydrocarbons.

TEST: The XRD analysis and EDS/SEM examination of sediments to look for secondary iron phases after reaction in both abiotic and biotic laboratory systems to which varying amounts of Fe (II) were added. Also, the analysis of the aqueous phase concentrations of Fe (II) to document changes suggestive of Fe (II) generation and loss, and loss of acetate, to document microbial activity.

### 6.3.2. Role of iron in the NATS Experiment

The NATS experiment was designed to create redox conditions that might produce changes in the iron-bearing minerals in the Columbus AFB aquifer. The amount of dissolved Fe (II) released to groundwater during the experiment indicated that iron minerals were reduced in the region near the NAPL source by concomitant oxidation of hydrocarbons leached from the source by groundwater. Since the local groundwater is low in dissolved nitrate and sulfate, microbial oxidation of the emplaced hydrocarbons by Fe (III) becomes the primary redox process after molecular oxygen is depleted. This study was directed to identification of possible changes in the Fe (III)-bearing phases due to microbial degradation at selected core locations downfield from the NAPL source. It was also intended to augment data collected by our NATS collaborators in assessing the capability of iron oxide phases to support degradation processes. It is emphasized that the amount of hydrocarbon in the NATS source was limited by regulatory agencies. It certainly would have been desirable to conduct the NATS experiment with at least a one order of magnitude larger hydrocarbon source, which would still be less than the content of one of the thousands of underground fuel tanks that have leaked into aquifers of the United States. With the relatively small amount of hydrocarbon emplaced and the high abundance of iron in the local aquifer material, large changes in iron mineral phases due to hydrocarbon oxidation were not expected, but it was hypothesized that changes in these minerals would be observed near the source. Testing of this hypothesis

and associated sub-hypotheses regarding particular redox reactions of site iron minerals is the subject of this thesis (Section 6.3.1).

Investigation of changes in iron minerals required chemical characterization, which was done primarily by analysis of total iron, amorphous iron, and of Fe (II) concentrations in extracts of site geological solids using selective extractants. Also the iron-bearing phases were examined by powder x-ray diffraction, and surface iron concentrations and morphology were assessed by scanning electron microscopy/energy dispersive spectroscopy.

### 6.3.3. Laboratory Microbial Oxidation Experiments

Laboratory experiments were conducted to investigate reactions of site aquifer materials and of iron-containing minerals with hydrocarbons under controlled environments. The first experiment was designed to give possible indications of reactions that might be observed in the NATS field situation. The second experiment was designed to examine formation of secondary minerals (e.g. magnetite) during the consumption of hydrocarbons as a function of dissolved Fe (II) concentration. Results of these experiments are of qualitative value, but cannot be extrapolated quantitatively to make field site concentration predictions, due to differences between the field and laboratory conditions that include:

1) There is high spatial heterogeneity of the aquifer sediment properties that may produce microzones of oxic and anoxic degradation that are separated by short distances. This situation does not apply to laboratory studies of homogenized samples.
2) Some hydrocarbons in the field NAPL were not used in laboratory experiments, and hydrocarbon concentration did not vary spatially in the laboratory batch experiments.
3) The field site is a flow through system, which was not replicated in the laboratory. Aqueous solutions of hydrocarbons and degradation products including Fe (II) will be "flushed" from the aquifer, unlike in the batch situation. Thus, concentrations of both products and reactants obtainable

in the laboratory batch systems are much higher than in the field, so that reactions observed in the laboratory may not be important at field sites. Laboratory flow-through microcosm experiments might resolve the importance of flushing, but were not done.

4) The pH of the field groundwater and the laboratory experiment solutions is different (~5.2 in the field and ~7 in the laboratory experiments). Thus, the solubility controls on secondary mineral will be different and minerals formed under laboratory conditions may not form in the field.

# 7. Methods of Analysis of Aquifer Materials

## *7.1. Details of Analytical Methods*

Analytical methods detailed below were employed to assess the chemical and physical changes in aquifer materials coinciding with microbial oxidation of organic materials. These methods included powder x-ray diffraction (XRD), specific surface area (SSA) analysis by nitrogen gas absorption, scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (SEM/EDS), gas chromatography, selective iron extractions, total iron analyses by flame atomic absorption (FAA) or inductively coupled plasma spectrophotometry (ICP), and Fe (II) analyses by complexation with ferrozine and analyses by UV/Vis spectrophotometry.

X-ray diffraction analysis:

Approximately 0.5 g of sample was ground with a corundum mortar and pestle to a fine powder, about the consistency of cornstarch, and loaded onto sample holders fitted with a zero background quartz plate. XRD analyses were done on the < 0.45 mm size fraction of all samples and on the fine fraction (< 15 um, separated by centrifugation methods) of a few samples. XRD data were collected on a Scintag $X_2$ ADS system using an iron anode, and a Peltier (solid state) detector typically over a 4 to 100° 2Θ range in 0.02° steps for 8 seconds/step. Background subtraction was performed using a manual three point spline method and peak identification was based on comparison to an ICCD(II) file. The expected pattern for amorphous iron was taken from

Schwertmann et al. (1982). Additionally, a small subset of samples were scanned with finer resolution over the primary iron oxide peaks to discern changes in these phases; data were collected over °2θ ≈ 43-47, for 1 to 2 days which improved signal-to-noise although the data were still "noisy".

Specific surface area analysis:

SSA is the mass-normalized surface area of a sample, expressed as $m^2/g$; small particles and porous particles tend to have higher SSA. SSA was determined using nitrogen gas absorption. Approximately 0.1g sub-samples of freeze-dried sediment were prepared in the Micromeretics flow-prep station by passing nitrogen gas through the sample while heating at 102°C for at least two hours. The sample was then capped, cooled and accurately weighed before the measurement of nitrogen adsorption using a Micromeretics Gemini 2375, balanced tube instrument. SSA was based on a 5-point nitrogen adsorption curve using the BET equation (Gregg and Singh, 1982). Before each day's analyses, the Micromeretics machine was calibrated to a known kaolinite reference standard (Standard # 004/16819/00 at 16.8 ± 0.8 $m^2/g$).

Scanning electron microscopy / energy dispersive x-ray spectroscopy :

SEM /EDS using an Amray scanning electron microscope and attached IXRF EDS was used to provide morphological and chemical data on the iron phases. The freeze-dried sediment sample was dispersed over an aluminum stub, attached by a glue tab adhesive, and the sample then coated with 20nm of gold-palladium to minimize charging during imaging. A SEM image was acquired

and 15 individual particles were randomly "tagged" for chemical analysis by EDS. Spectral analysis data was collected for each of the tagged particles, and the iron to silica ratio of the particle was determined using an internal calibration.

Gas Chromatography (GC) analysis of acetate:

Concentrations of acetate were quantitatively determined on a Varian 100 gas chromatograph. Acetate samples were acidified by adjusting the pH of the sample to at least 1 pH unit below the $pK_a$ of acetate, 4.98. The polyethylene glycol ester column used for volatile fatty acid analysis can be damaged by repeated exposure to low pH solutions, so care was taken to not acidify the solution to below pH 3. The injection port and flame ionization detector were heated to 220°C, the column was kept at a constant 180°C, and the helium carrier was ~8cps. Note that although one set of laboratory experiments used toluene as the hydrocarbon source, toluene concentrations could not be accurately determined by GC or other methods because of the potential loss of the volatile toluene to the headspace in these experiments.

Selective Extraction of Iron from aquifer sediments:

The fraction of iron present in amorphous phases was determined by comparison of selective extraction for amorphous iron phases and complete extraction of iron phases. Additionally, the distribution of iron between redox states Fe (II) and Fe (III) in the amorphous phases was calculated (see details below). The total iron extractions do not preserve the initial redox state of the

extracted iron, and thus the total Fe (II)/Fe (III) ratio of the sediment iron oxide phases could not be determined.

Amorphous iron, constituting both Fe (II) and Fe (III), was extracted with 1 N HCl. (as modified from Chao and Zhou, 1983). Specifically, 0.25g sediment and 20 ml $N_2$-sparged 1N HCl were sealed in centrifuge tubes with the headspace filled with $N_2$. The samples were then periodically agitated in a water bath for 30 minutes at 80°C. Alternatively, for some of the extractions, the sediments and acid solutions were allowed to equilibrate in an anaerobic glovebox before the reaction. The sediment and acid were then sealed in serum vials with Teflon coated septum, brought out into the laboratory, and placed in the water bath. By performing these extractions under $N_2$, the redox distribution of amorphous Fe is preserved for analysis (see below).

Because Fe (II) comprises a small fraction of the total Fe in oxidizing aquifers, total Fe and ferric iron concentrations are similar. Fe (III) oxides were determined by a single extraction of 0.25 g sediment with 20 ml of 5 N HCl for two weeks at room temperature (Heron et al., 1994). While this extraction procedure is designed to attack Fe (III) oxides, the Fe (II) phases are also dissolved.

Iron Analysis:

The supernatants from the various extraction procedures were analyzed to determine the amount of Fe (II) and Fe (III) released. Fe (II) was determined by complexing 2 ml of extract supernatant with 2 ml of 0.004 M ferrozine buffered with 0.05M N-[2-Hydroxyethyl]piperazine-N'-2[ethanesulfonic acid] (HEPES) and measuring absorbance at 562 nm on a Shimadzu UV/Vis spectrophotometer. Fe (II) was only analyzed for the 1N HCl extractions (i.e. only for amorphous iron). The 5N HCl extract would overwhelm the buffering capacity of the ferrozine solution, so it was not subjected to Fe (II) analysis. Total iron was determined for all extractions (1 N HCl under N2 and 5N HCl) by ICP (using a Thermo Jarrel Ash Tracescan) or by FAA (using a Perkin Elmer AAnalyst 800). Amorphous Fe (III) is calculated as the difference between the Fe (II) and total iron concentrations determined in the IN HCl extracts.

### *7.2. Considerations regarding analytical data on aquifer material samples*

NATS core samples

Aquifer material core samples collected by our collaborators during NATS were analyzed by several methods in order to identify possible changes in the iron phases due to microbial iron-reduction. The core samples were characterized by XRD, acid extraction of iron in various forms, SEM/EDS, and SSA analysis. While each method provides important information, results from several methods of analysis were combined to present stronger evidence of the possible changes in the iron phases of the aquifer material. Grain size effects on data for samples from this heterogeneous aquifer were minimized by conducting all the analyses on particles obtained by sieving through a 0.45mm mesh screen.

Matching the diffraction patterns gathered by XRD to a library of known diffraction patterns identified crystalline phases of the aquifer material. A semi-quantitative analysis giving relative concentrations was obtained by comparing the areas of the primary diffraction peak of each crystalline phase found. Changes in the relative abundance of iron phases in the aquifer may be represented by the changes in the ratio of the areas of the primary peak of that iron phase and the primary quartz peak resulting from the ubiquitous quartz. A decrease over time in this ratio for samples from a particular site location represent a probable decrease in the concentration of that iron phase over time. For one nearest field location at mid-depth (i.e. the region of the aquifer that had

the greatest dissolved aqueous indicators of microbial activity), detailed narrow scans over a main Fe oxide peak ($2°\theta \approx 44.4$) also were collected in order to observe any changes in the Fe oxide content of the sediments. This peak position is not the primary diffraction peak for goethite, but more intense goethite peaks are obscured by quartz peaks that dominate the diffraction pattern.

The total abundance of all iron phases was determined by acid extraction of core samples; these extractions were performed on samples from each sampling period in order to monitor changes in the iron mineralogy over time. The January 1996 aquifer samples were used as a baseline for iron-phase extraction analysis. Since the hydrocarbon plume had not yet reached the near field sampling locations by January 1996, variability in their extract concentrations is representative of the heterogeneity of the aquifer material. The variability of the iron-phase concentrations in January 1996 samples was as high as variations observed at the same location over time (within the limitation that same sample location can not be cored repeatedly). Thus, high local heterogeneity caused the iron phase extraction methods to be useful primarily for documenting the presence or absence of the iron-phases, and not necessarily for assessing changes or trends in iron phase abundance.

Important morphological and chemical data were obtained by SEM/EDS. Randomly chosen particles from each core sample were analyzed for their elemental concentrations. Iron to silicon ratios were averaged for all the particles tagged in a given sample. Comparison of these ratios among samples collected

from the same area in the aquifer but at different times allows for the estimation of changes in the abundance of iron phases during the NATS experiment.

Changes in the SSA of aquifer sediments collected from the same area also were assessed. The change (increase or decrease) depends on the forms of the Fe (III) phases consumed and of any secondary phases formed by microbial activity. A change in SSA with time may indicate microbial use of Fe (III) phases. For example, if Fe (III) forms high surface area coatings over most of the sediment particle surface, loss of the Fe (III) oxide coatings may produce lower SSA, as the underlying quartz (of much lower SSA) is exposed. Alternatively, if the Fe (III) phases are located primarily in cracks and other high SSA surface defects of the underlying quartz particles, consumption of Fe (III) may result in an increase in SSA of the sediment. Additionally, secondary phases with distinct but unknown (likely high) SSA may form. In summary, we may see a change in SSA upon loss of incipient Fe (III) phases but cannot predict the magnitude or direction of that change without additional information on the form of the Fe (III) phases present.

Laboratory Experiment Solids Characterization

Solids from the microbial growth and magnetite formation laboratory experiments were analyzed by the same methods as used for the site aquifer material samples. Changes in the iron mineralogy were assessed by the changes in the relative ratios of peak areas from XRD analyses and from the silica and iron ratios from SEM/EDS analyses. Additionally, the supernatant from

the microbial growth laboratory experiments was monitored for acetate and toluene oxidation and Fe (II) production.

For the magnetite experiment, similar solids and solution analyses were performed. As for the microbial experiments, the aqueous Fe (II) and acetate analyses were used to document microbial activity. The Fe (II) content of the solids was also determined to assess Fe (II) sorption. The XRD data provided insight into the type of secondary phases formed under abiotic and biotic oxidation of organics. EDS/SEM allowed for identification of morphological differences in the secondary iron phases, in particular of the magnetite.

### *7.3. Statistical Treatment of Data*

Field Samples

Single cores were collected at three depths from each sampling location at each sampling date; cores were also periodically taken from the source region. Only single cores were collected because of the high costs of coring and the need to minimize disturbance of the aquifer (see Section 8.1). Subsequent cores from the "same" sampling location were separated laterally by up to 1 meter to minimize effects of disturbance of the aquifer by prior coring.

For each homogenized core, two subsamples were analyzed by the various methods described. As noted above, these subsamples were sieved to remove particles > 0.4 mm in order to minimize particle size effects on the results. Analyses for the duplicate subsamples were averaged, and the Standard Mean Distribution (SMD) (Zarr, 1996) calculated as follows:

$$SMD = \frac{\sum|\chi_i - \overline{\chi}|}{n}$$

Both the average value and, where appropriate, the SMD are provided in the presentations of results.

In order to assess the inherent heterogeneity of the aquifer, the distribution of Fe extraction values for the January 1996 sampling (36 samples total: 3 depths for each sample, 3 samples per zone, and 4 zones) was determined over all 36 cores (See Appendix, Figures A1 – A4). The January 1996 cores were taken prior to significant migration of the contaminant plume, thus represented background (before contaminant loading) conditions of the aquifer. Both of the bulk iron measures (i.e., total Fe, and amorphous Fe (III)) were found to have a lognormal distribution (Total Iron mean = 0.367 (w/w%), standard deviation (+0.448, -0.202); Amorphous Iron mean = 0.094, standard deviation (+0.082, -0.044). The standard deviations of these means are skewed due to the log transformation. These means and standard deviations are consistent with previously published measures of heterogeneity of site aquifer material organic carbon content, specific surface area and hydraulic conductivity, which also had lognormal distributions (MacIntyre et al., 1998). Analytical results for cores collected during subsequent sampling times, and that are outside the standard deviation of the mean are considered to depart significantly from the natural heterogeneity of the aquifer.

Laboratory Samples

The microbial experiments used a single source of homogenized sediment from the NATS site in order to overcome the inherent heterogeneity of the field samples. For the experiments using the natural microbial population (also from the NATS site), no additional sources of iron were included. However, GS-15 (*Geobacter metallireducens*) is cultured on amorphous iron hydroxide and cannot be sustained in its absence; thus an unknown quantity of iron (III) hydroxide was unavoidably introduced to each incubation vial containing GS-15. Care was thus taken in comparison of data from GS-15 experiments with those using the natural microbial consortium.

Each set of conditions ("system") for both the microbial and magnetite experiments was established in triplicate. Individual (single) analyses of the supernatant and sediments from each of the three vials were averaged, and the coefficient of variation (Zarr, 1996) determined as follows:

$$CV\% = \frac{Std.Dev.}{\bar{\chi}} * 100$$

Both the average values and percent coefficient of variation are reported. For those systems with less than three samples (due to breakage of vials) the SMD is given. The coefficient of variation for each analysis is given along with the mean for each analysis.

## 8. Iron Bearing Minerals in NATS

This research was designed to investigate possible changes in abundances of iron-bearing minerals of the Columbus AFB aquifer due to microbial iron (III) oxidation of the emplaced hydrocarbons, particularly in near

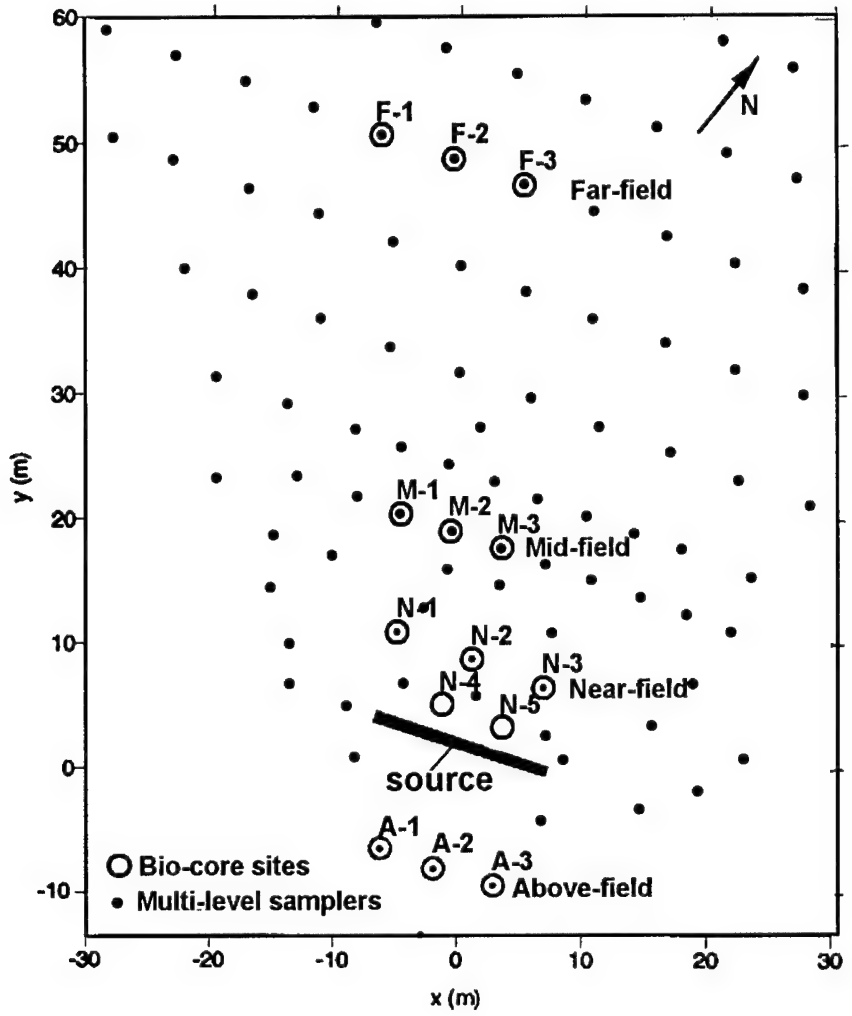
source regions. In order to document possible changes in iron minerals, aquifer material core samples were taken at several times and locations during the NATS experiment.

### *8.1. Sampling methods and locations*

Cores located on the four transects shown in Figure 4 were taken at several times during each year of the study. Coring was done using a truck mounted auger drill to remove material to the depth of the top of the desired core, and a split barrel core tube with sterile plastic liner was then driven to appropriate depth with a hydraulic hammer. The cores are referred to in Figure 4 as "Biocores" because contents of each core sample collected were divided into a portion for microbial and gene probe analysis at the University of Tennessee and a portion for the iron mineral analysis reported here. The four sediment core transects are defined as: above field (A1-A3), 10 meters up-gradient from the source; near field (N1-N5), 2 to 10 meters down-gradient from the source, mid-field (M1-M3) and far field (F1-F3), 20 meters and 50 meters respectively down-gradient from the source (Figure 4). Three cores were taken at each location, at approximately 4, 6 and 8 meters below the surface, denoted as "top," "middle," and "bottom," respectively. The two very near field locations (N4 and N5) were added after the start of the experiment because of observed near field changes in the aqueous chemistry; therefore, there are no baseline data for these

locations (i.e. no Jan 1996 data). Cores with the same sample designation but taken at different times were generally within 1m laterally, as noted above. The number of cores was limited by coring cost, and by the constraint of minimum aquifer disturbance (including addition of atmospheric oxygen). Also, this requirement for minimum disturbance precluded otherwise desirable dense core spacing in the near source region. In summary, it is re-emphasized that replicate (repeated) coring at any spatial-temporal location in the aquifer was precluded by cost and aquifer disturbance constraints, and that cores at the "same" sample location, at different sampling times, were not exactly co-located, but were adjacent, and thus subject to local heterogeneity of the aquifer material.

**Figure 4: Location of Sites for Both Water Samples and Sediment Cores**


F-1
F-2
F-3
Far-field
N
M-1
M-2
M-3
Mid-field
N-1
N-2
N-3
N-4
N-5
Near-field
source
A-1
A-2
A-3
Above-field
Bio-core sites
Multi-level samplers
y (m)
x (m)
60
50
40
30
20
10
0
-10
-30
-20
-10
0
10
20
30

Upon collection, each core sediment sample was immediately placed in a glass vial and frozen to limit oxidation of Fe (II). The samples were then shipped to the VIMS laboratory on ice by overnight mail. Upon arrival, they were stored at -80°C until processing, which consisted of freeze-drying and sieving to remove particles larger than 0.45 mm. This homogenized, <0.45 mm fraction was stored at room temperature, and was used for all the analytical characterizations described above.

The aquifer was cored at five times during the NATS experiment: January, April and September 1996, and March and September 1997. After completion of NATS, additional coring for plume observation was done in 1998 and in 1999. Coring dates were determined, in part, by groundwater concentration data collected by other NATS investigators. These data included pH, dissolved oxygen, total organic carbon, aqueous Fe (II) and methane concentrations, and $^{12}C/^{13}C$ isotope ratios in groundwater near the coring sites (see Appendix, Table A1 for selected data).

The January 1996 samples provided the background data to which all other samples were compared[1]. After this time, a distinct, migrating hydrocarbon plume was observed with the highest concentrations near the middle core depth. Core samples from this depth were deemed the most likely to show changes in the iron-bearing phases because of their enhanced contact with the hydrocarbon plume; most of the subsequent samples were analyzed at this depth. The

[1] This is justified because the hydrocarbon plume had not migrated significantly down gradient in the 40 day period from the emplacement to the sampling date in January 1996.

samples analyzed are outlined in Table 2. Note that the April 1996 samples were compromised by improper handling by field personnel, and could not be used for this study.

### *8.2 Analytical Methods*

The sediment samples collected in the field were analyzed by several methods to determine their iron mineralogy. Selective iron extraction, Fe (II) analyses, x-ray diffraction, and SEM/EDS analyses were performed on the samples listed in Table 2. These methods are described in Section 7.1.

## Table 2: Core Samples for CAFB Aquifer Characterization Analysis

Samples were taken from 4 meters (T = top level), 6 meters (M = middle level), and 8 meters (B = bottom layer) below grade. A full suite of analyses was performed on samples denoted by S. Samples were denoted by E were kept frozen for future analysis. Selected analyses (but not all) were performed on some of these samples. Locations are denoted as A for above source, N for near source, M for mid-field, and F for far field. The source was located at the middle level.

| Sample Name | Jan. '96 | Sept. '96 | March '97 | Sept. '97 |
|---|---|---|---|---|
| | T M B* | T M B | T M B | T M B |
| A1 | S S S | E S E | E S E | E S E |
| A2 | S S S | E S E | E S E | E S E |
| A3 | S S S | E E E | E E E | E E E |
| N1 | S S S | E S E | E S E | E S E |
| N2 | S S S | E S E | E S E | E S E |
| N3 | S S S | E E E | E E E | E E E |
| N4 | | S S S | S S S | S S S |
| N5 | | S S S | S S S | S S S |
| M1 | S S S | E S E | E S E | E S E |
| M2 | S S S | E S E | E S E | E S E |
| M3 | S S S | E E E | E E E | E E E |
| F1 | S S S | E E E | E E E | E E E |
| F2 | S S S | E S E | E S E | E S E |
| F3 | S S S | E E E | E E E | E E E |
| Source Trench | | | S S S | S S S |

### *8.3 Results*

The analysis results for the field samples should be interpreted in light of the high heterogeneity of the aquifer (see discussion in Section 7.3). Unfortunately, it was not possible to take enough cores to measure geostatistical heterogeneity of iron mineralogy in the detail done for other site aquifer properties by MacIntyre et al. (1998).

XRD data provided a qualitative analysis of the crystalline solids in the aquifer, and indicated that all bulk samples and fine-fractions are predominantly quartz. Minor clays (illite and kaolinite) and iron oxyhydroxide (goethite) were detected in many of the bulk samples. In addition, an exchangeable clay (smectite) and amorphous hydrated ferric oxide (HFO, or ferrihydrite) were detected in the fine fractions, indicating that there are trace to minor amounts of these reactive phases in the aquifer materials. Quantification of the iron oxide minerals present by XRD is difficult because the prevalent small crystallites of iron oxides (i.e. cryptocrystalline goethite) produce broad diffraction peaks; thus these minerals can be identified but not readily quantified with XRD. The situation is exacerbated for amorphous materials that produce broad low peaks that usually cannot be identified in XRD data for aquifer material samples. Also the iron minerals are generally a small percentage of the total sediment, making their identification and quantification difficult.

Iron mineralogy was also examined by more detailed XRD analysis (smaller steps and longer counting times) of the principal iron oxide peak

separate from the quartz peaks, in particular for aquifer material from sample N-4, mid-level, the location and depth where aqueous chemistry data showed the greatest evidence of microbial activity. First, the expected changes in this region of the XRD pattern were discerned by comparing sediment as-received and sediment with all iron oxides extracted. Figure 5 shows that the broad peak at 44.4° 2Θ is due to extractable iron. Similarly, there is an apparent decrease in iron content over time at this location. The broad peak 44.4° 2Θ has largely disappeared in the March 1997 sample relative to earlier sampling dates, implying removal of the available iron. Furthermore, these results suggest that the microbial community uses crystalline iron oxides and is not limited to amorphous iron oxide.

Amorphous iron concentrations of the sediment determined from the 1N HCl extraction are given in Figures 6 – 9 and Appendix Table A2. The detection limit (0.001 w/w%) was determined as three times the standard deviation of the blanks, converted to a w/w% using the average weight of a sample (0.25g) and the volume of the extract (0.02L), this is well below the sample values. In most areas of the aquifer, the data show that the sediments are heterogeneous. Additionally, the values for the last sampling time, Sept 1997, are anomalously low for most samples including in the far field; this indicates that the extraction of amorphous Fe from these samples may have been incomplete. The samples from Sept. 1997 were not used in these determinations. Thus, it is difficult to ascertain temporal changes in the amorphous iron content of the sediments. Nonetheless, it appears (Figure 7) that there is an *increase* in amorphous iron at

the mid-depths of the nearest field samples (N-4 and N-5) between Sept 1996 and March 1997. These data also suggest mobilization of Fe (II) from within and/or nearer to the source and subsequent precipitation of new iron phases. Note that other sample locations also have increases in total amorphous Fe, including above source locations, so these data are not conclusive. However, the N-5 middle level increase in total amorphous Fe in March, 1997 is accompanied by a *decrease* in amorphous Fe (II) (see Figure 13 and Appendix Table A3) which supports the argument that this increase in total amorphous Fe is at least in part due to formation of new iron phases; at all other locations, increases in total amorphous Fe have a corresponding increase in amorphous Fe (II).

The Fe (II) content of the sediments, given in Figures 10-15 and listed in Appendix Table A3, also shows that the samples from the field are heterogeneous although in general they are very low in Fe (II). The detection limit (0.05 ppm), determined by three times the standard deviation of the blanks, was well below the sample values. Sediment samples from section N-5 had Fe (II) concentrations that were approximately two times higher than seen in the rest of the aquifer samples. Ferrous iron released from microbial activity is non-conservative, i.e. it may be removed from the groundwater via sorption processes. Thus, increases in Fe (II) may be an indicator of Fe (II) release from upgradient microbial degradation processes and subsequent resorption onto sediment phases. However, this can only occur appreciably near the site of Fe

(II) release, that is, before transport of Fe (II) in groundwater to oxygenated regions where it is precipitated as Fe (III) oxides.

Total iron concentrations, determined from 5N HCl extractions, showed the heterogeneous nature of the aquifer (See Figures 16-20 and Appendix, Table A4). The detection limit ($9.0 \times 10^{-5}$ w/w%) was determined as three times the standard deviation of the blanks, converted to a w/w% using the average weight of a sample (0.25g) and the volume of the extract (0.02L); this is well below the sample values. In general, the field samples did not show any consistent decrease in iron concentration except in the near field at N-2 (middle level), N-4 (top and middle levels) and N-5 (bottom level) (see Figures 17 & 18, and Appendix, Table A4). Note that samples from middle level N-4 also showed a decrease in crystalline iron in the XRD patterns (Figure 5).

The EDS data, listed in the Appendix, Table A5, shows a decrease in surface iron concentrations in the near field, which probably is not significant due to the large CV%. Detection limits could not be determined for these analyses. The Fe/Si ratio is determined by an internal standardization procedure for the instrument and software. The Fe/Si ratio was always lower in the final (Sept 1997) samples than in the preceding sample, and is at its lowest value in six of these eight samples (N-2 and N-3 Middle levels, and N-4 and N-5, Top, Middle and Bottom Levels). One of these sampling locations (N-2) was not analyzed for the middle sampling times (Sept 1996 and March 1997). However, of the remaining seven samples/locations, the Fe/Si ratio *increased* for these middle sampling times, with the Sept. 1997 final samples having a two to five fold

decrease in Fe/Si compared with the March 1997 samples. This increase may reflect mobilization of Fe (II) from material even closer to the source zone by to microbial reduction, with subsequent reprecipitation in the near field. Thus, "upgradient" microbial activity may result in an increase in hydrocarbon oxidizers (i.e. ferric iron phases) in the downfield. The EDS data also illustrate the heterogeneity of the aquifer, as the initial Fe/Si ratio, i.e. that for all samples taken in January 1996, ranged from 0.88 to 1.65 with an average of 1.13.

Temporal variation of the average concentration of total iron in the source material shown in Table 3 reflected the heterogeneous nature of the source material. There was no decrease in total iron over time, as shown in Figure 21. No change was expected as the source material was clean, washed sand, chosen to be an inert substrate for the hydrocarbon emplacement.

**Figure 5: XRD Pattern Over the Dominant Fe (III) Oxide Peak at ≈ 44.6 °2Θ for Sediments from the Near Field, Middle-depth (MW-10).**

(a) Comparison of XRD data for as-received and with iron oxides removed, September 1996 samples. (b) Comparison of samples collected from the same area over time. Note: XRD data are raw, box-car filtered data, calibrated by the 46.405 °2Θ quartz peak.

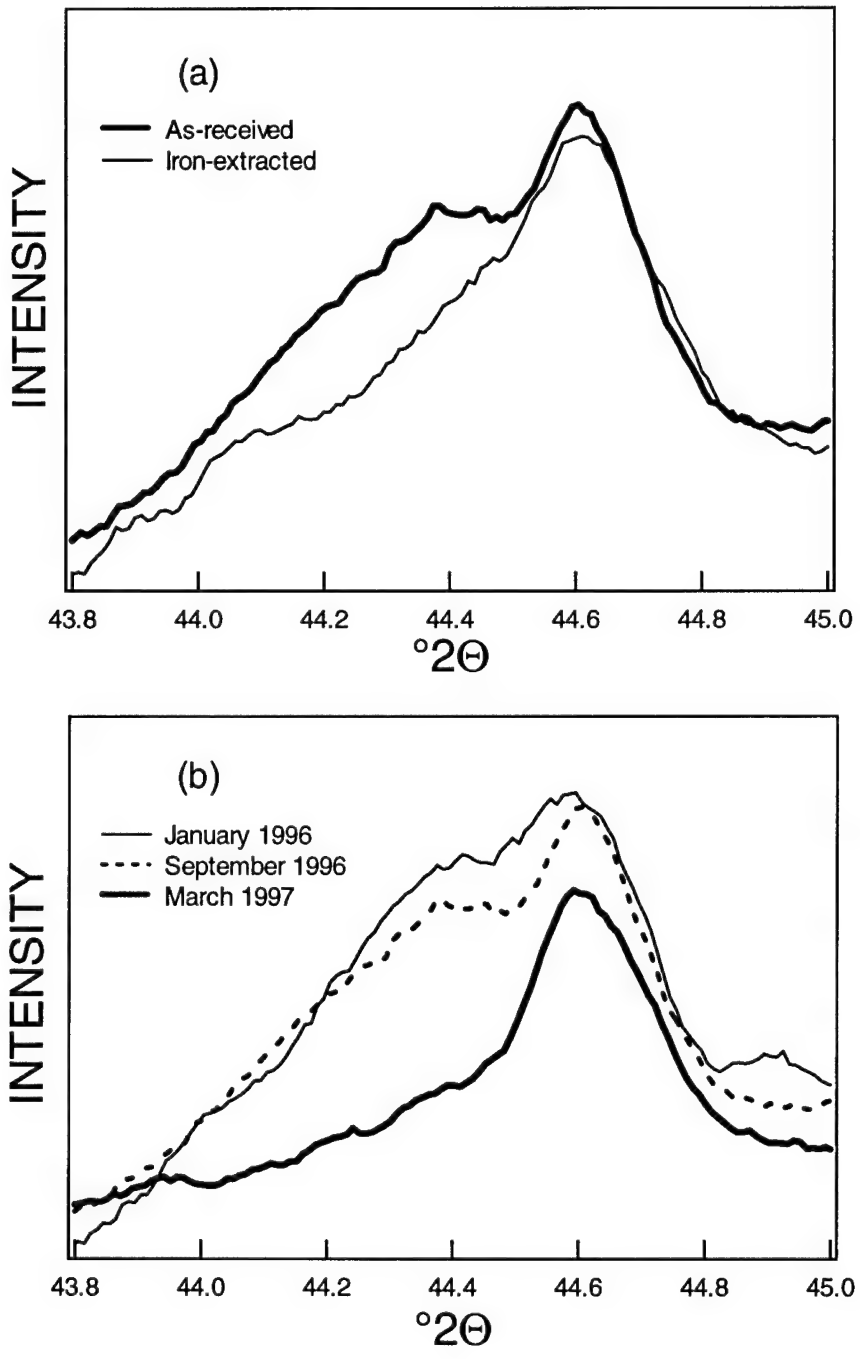


**Figure 6: Amorphous Iron (w/w %), Above field, Middle Level**

(Samples are the average of 2 analyses)

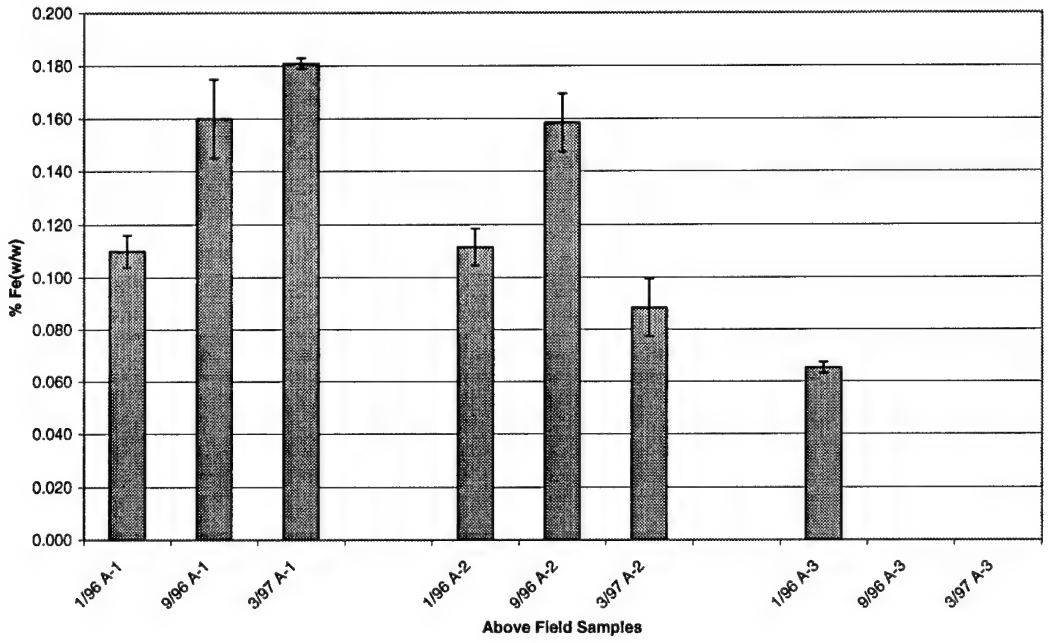


**Figure 7. Amorphous Iron (w/w %) in the Near Field Middle Level**

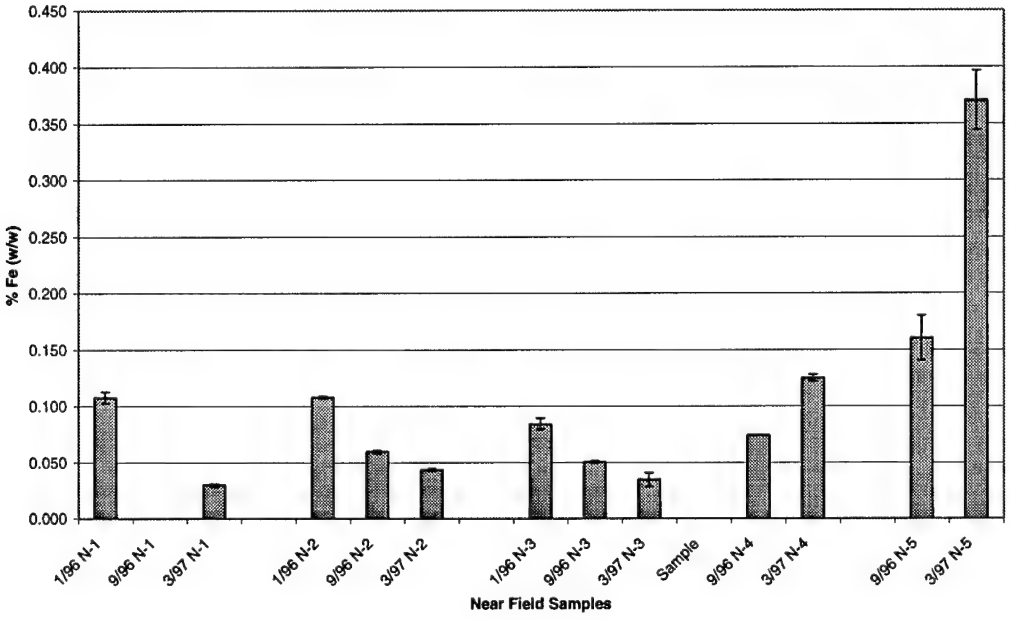
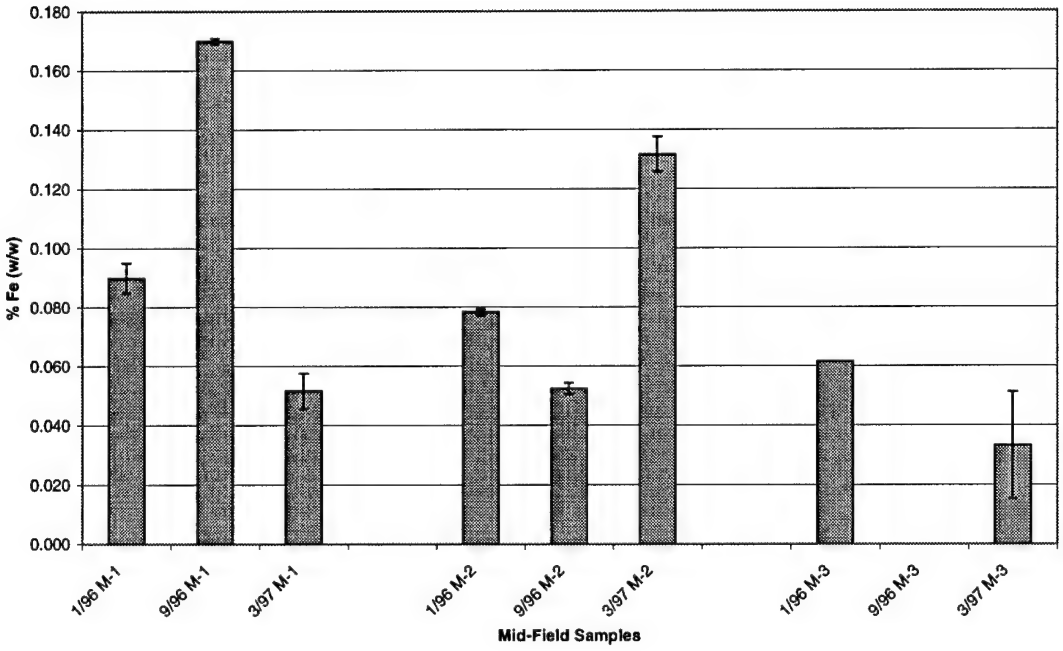


**Figure 8. Amorphous Iron (w/w %) in the Middle Field, Middle Level**



## Figure 9. Amorphous Iron (w/w %) Nearest the Source, All Levels

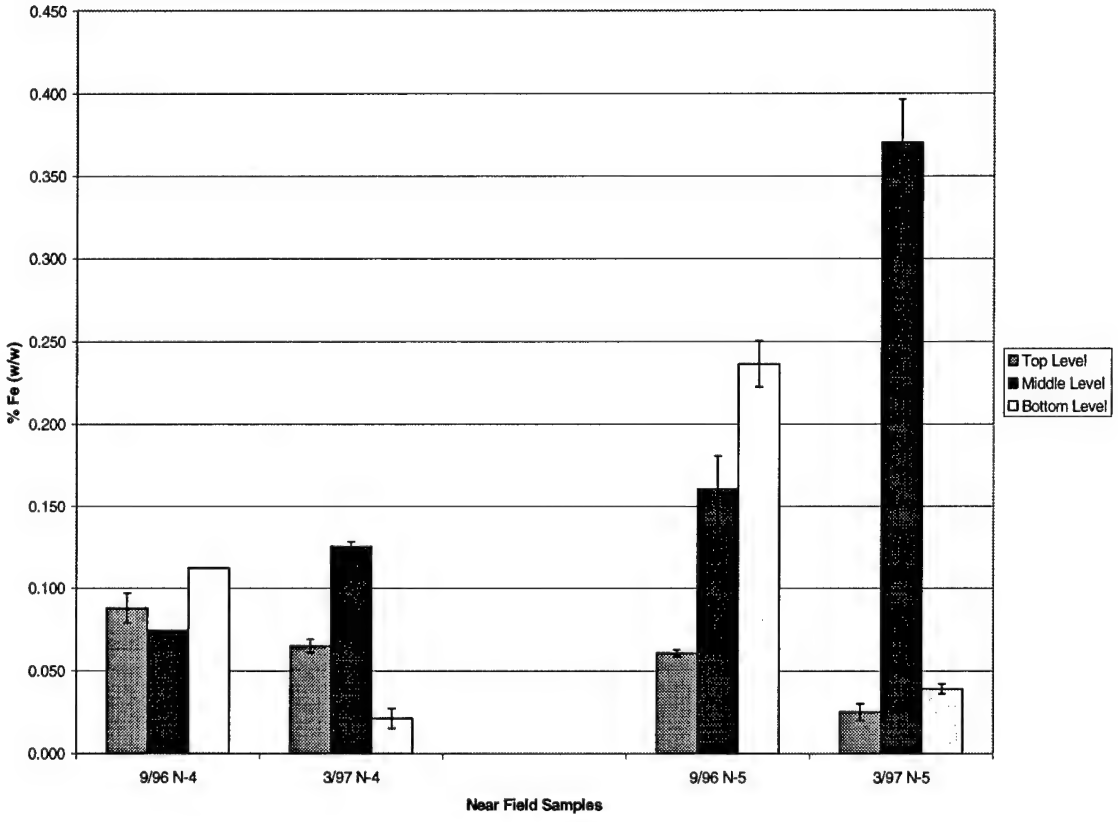


**Figure 10. Amorphous Iron (w/w%) in the Far Field, Middle Level**

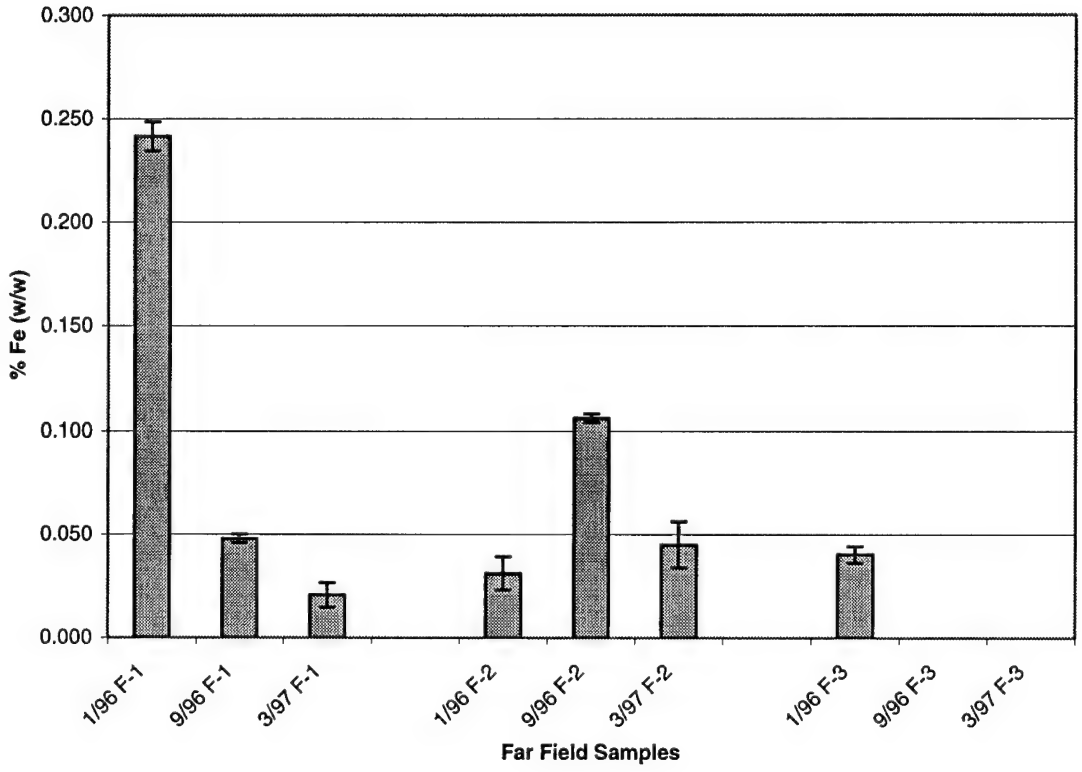
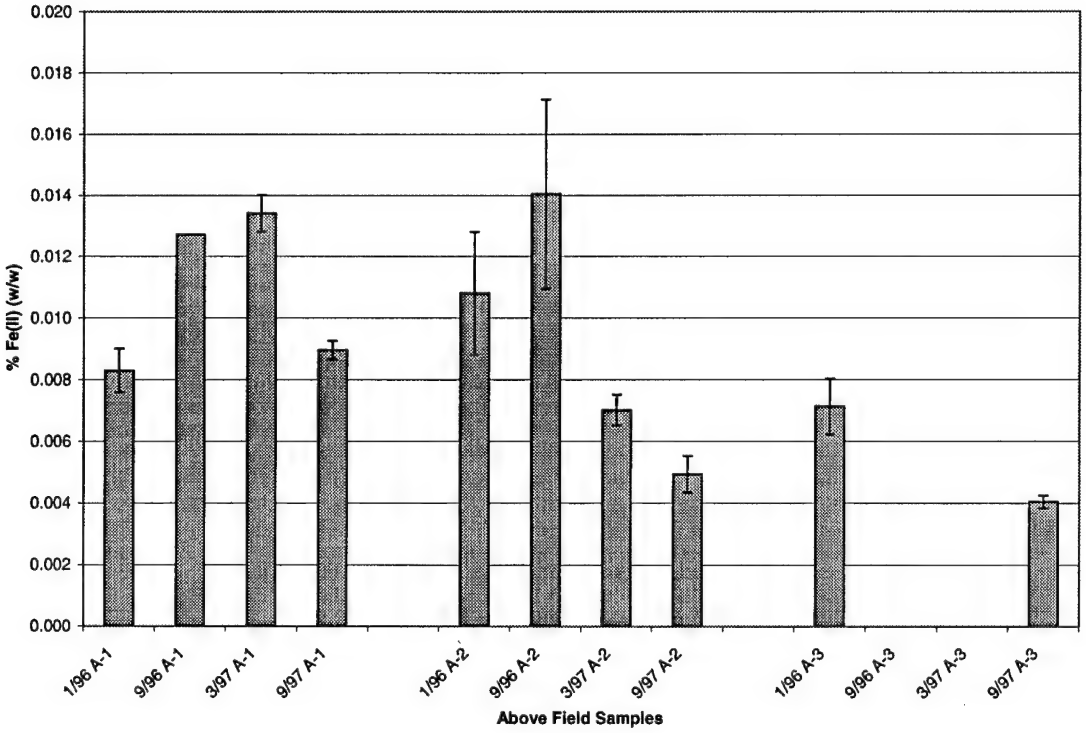


**Figure 11. Fe (II) (w/w %) in Above Field , Middle Level**



## Figure 12. Fe (II) (w/w %) in Near Field, Middle Level

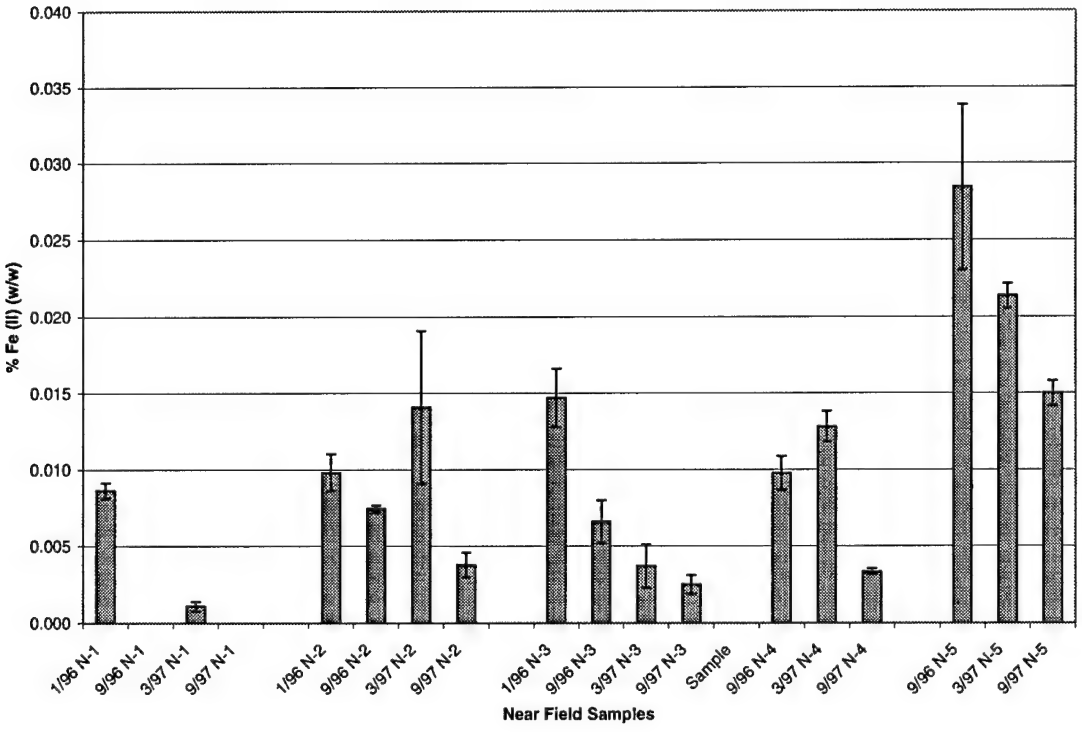


**Figure 13. Fe (II) (w/w %) in Cores Nearest to Source, All Levels**

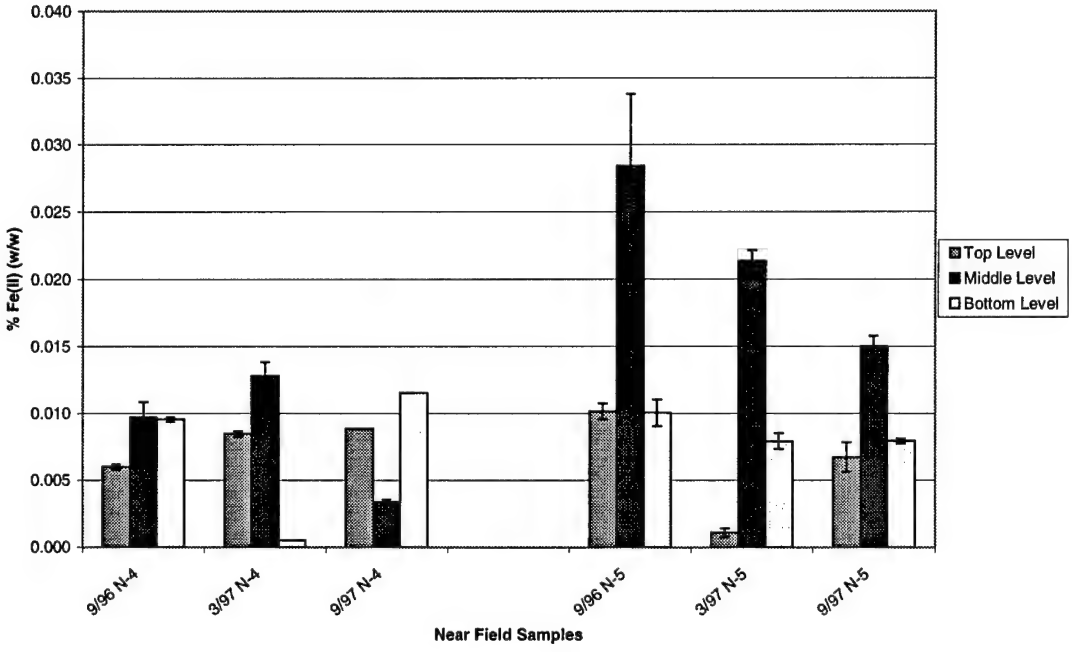
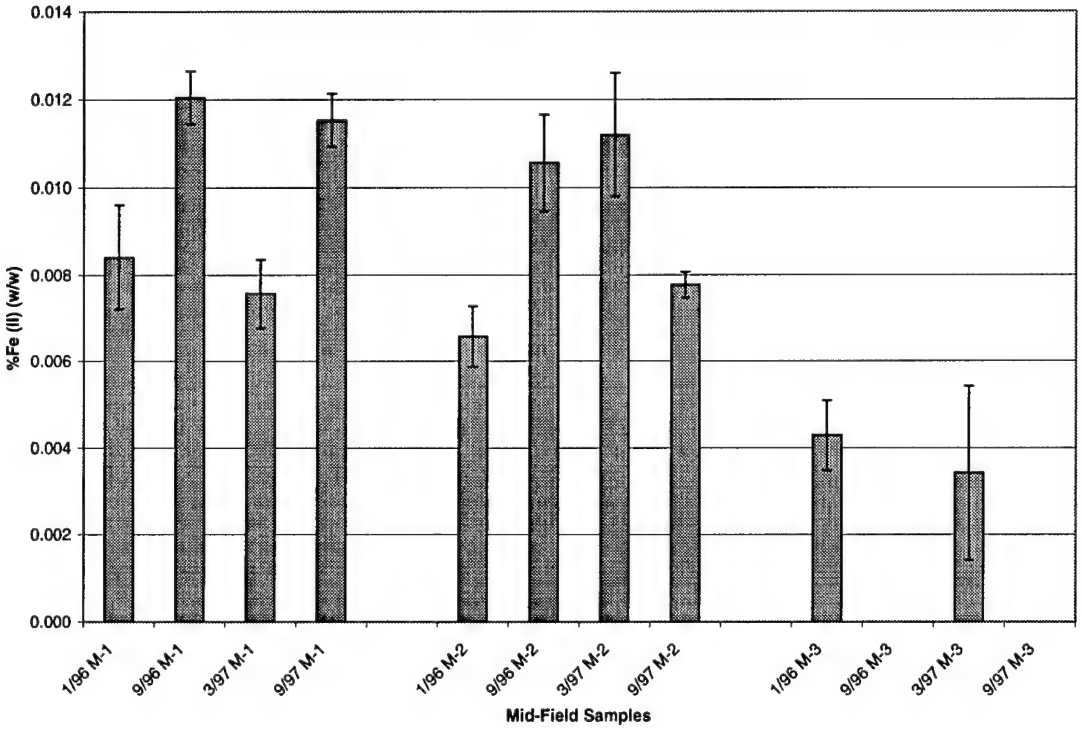


**Figure 14. Fe (II) (w/w %) in Mid-Field, Middle Level**



## Figure 15. Fe(II) (w/w %) in Far Field, Middle Level

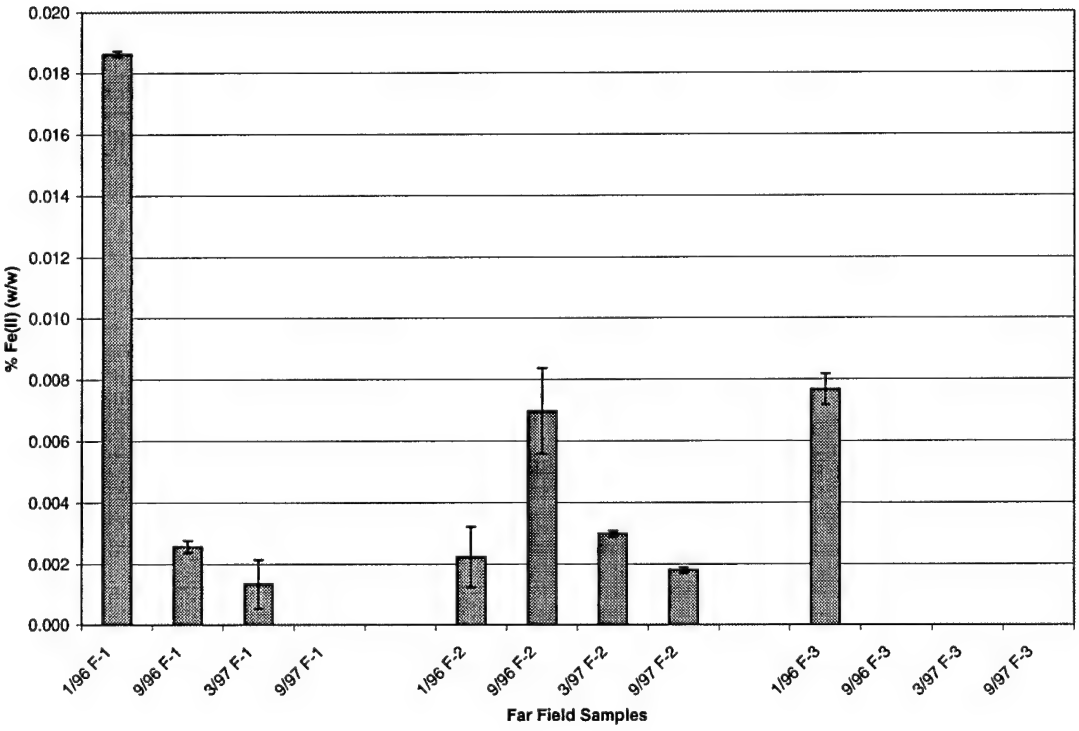
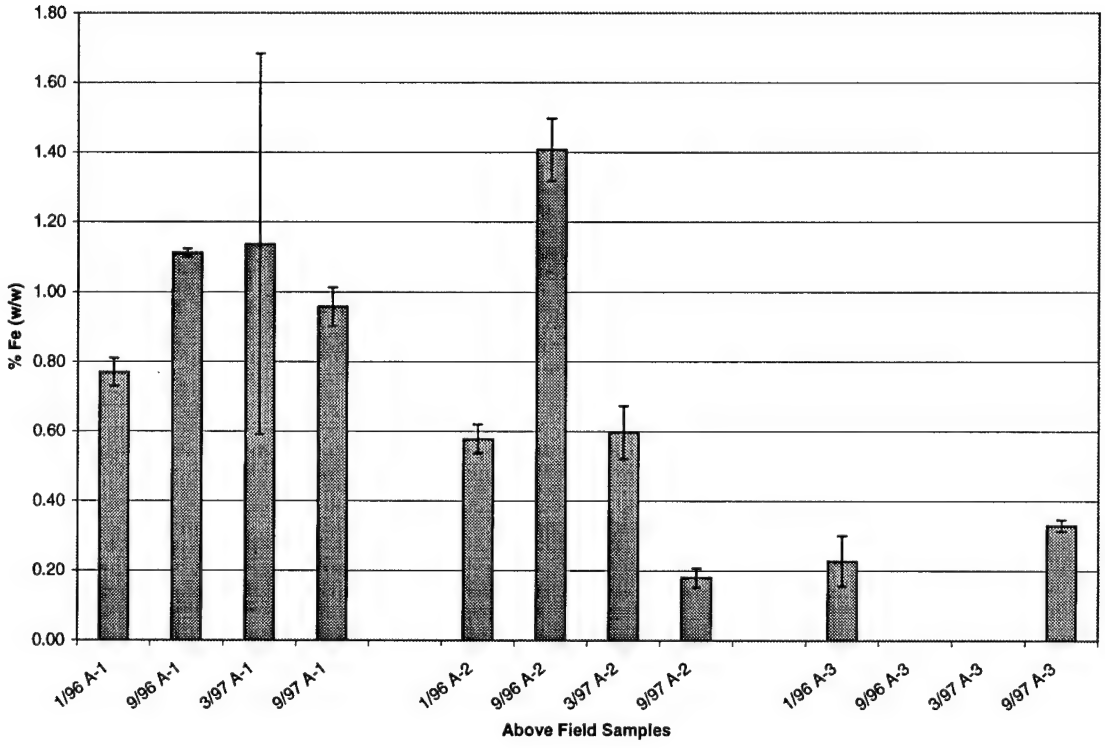
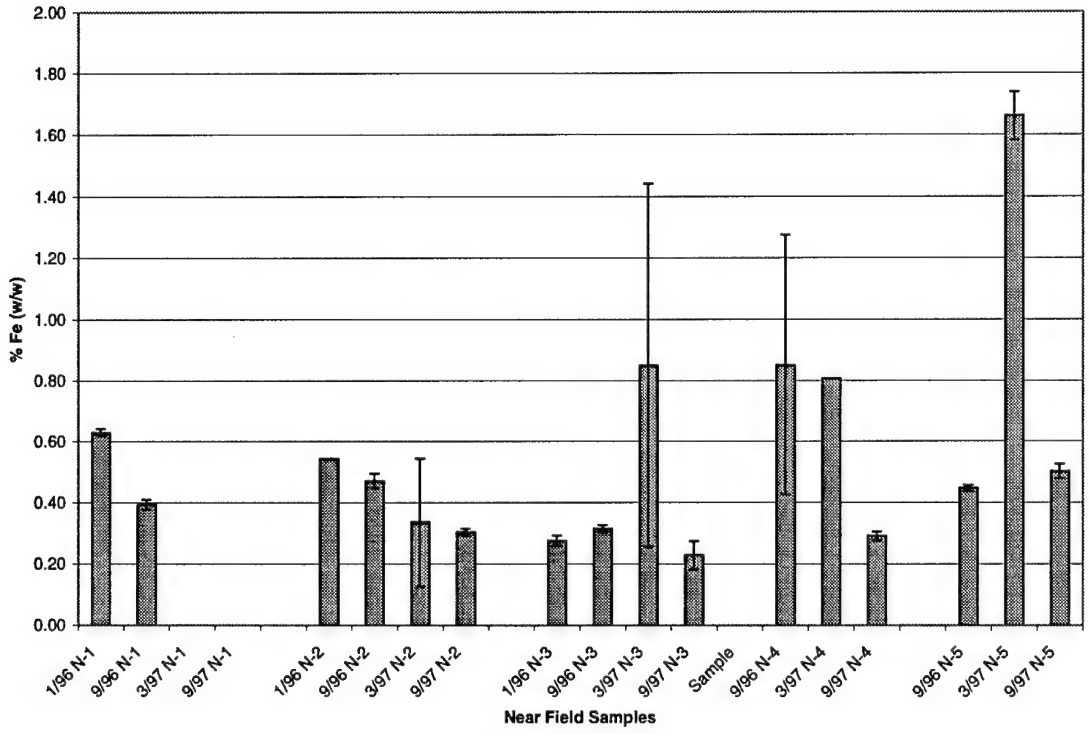


**Figure 16. Total Iron (w/w) in the Above Field , Middle Level**



## Figure 17. Total Fe (w/w %) in the Near Field, Middle Level



## Figure 18.Total Iron (w/w%) in Cores Nearest the Source

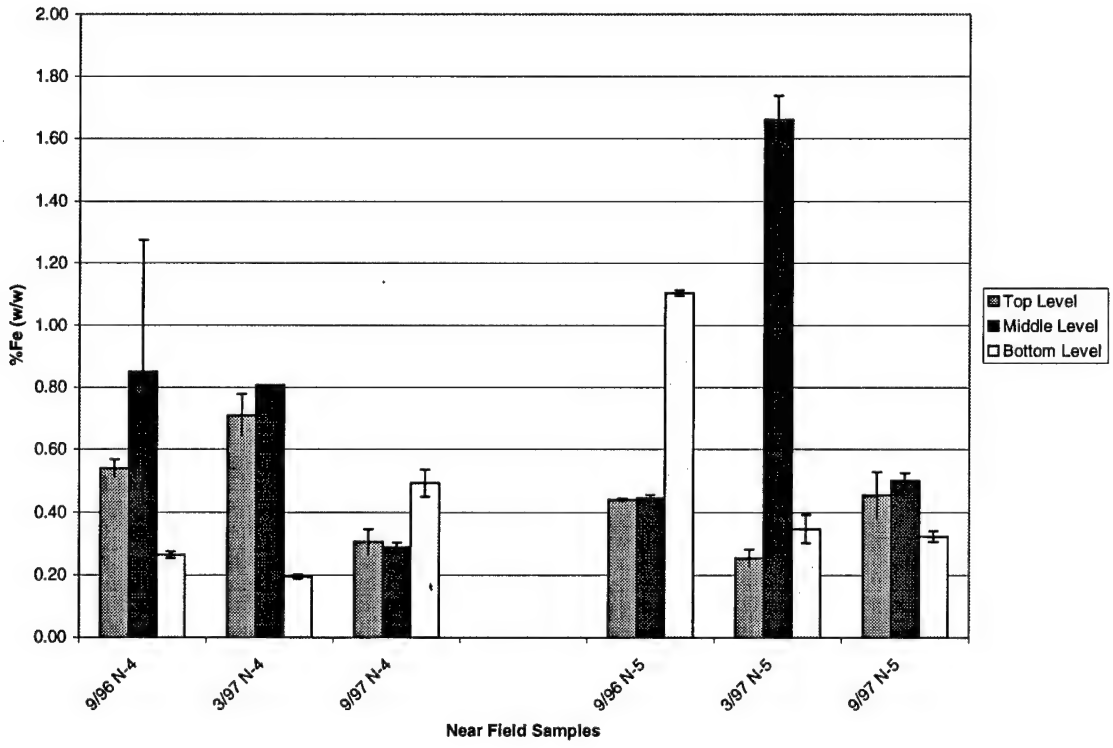
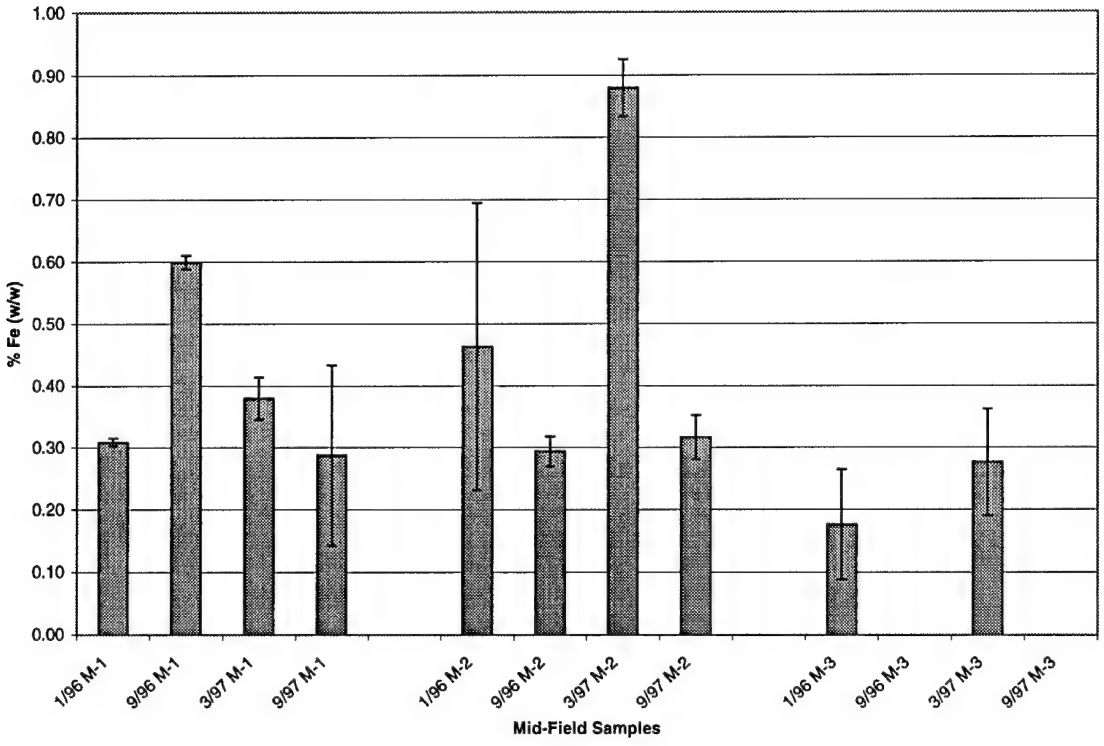


**Figure 19 Total Iron (w/w %) in the Mid-Field, Middle level**



## Figure 20. Total Iron (w/w %) in the Far Field, Middle Level

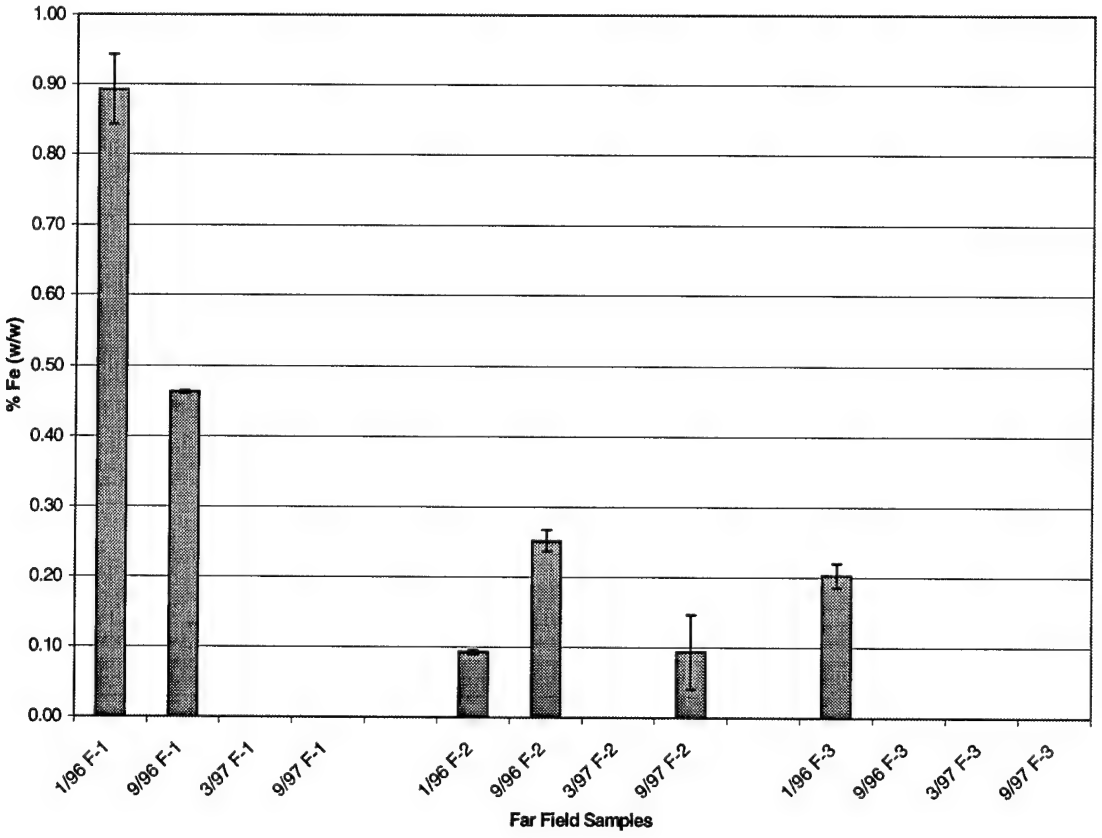
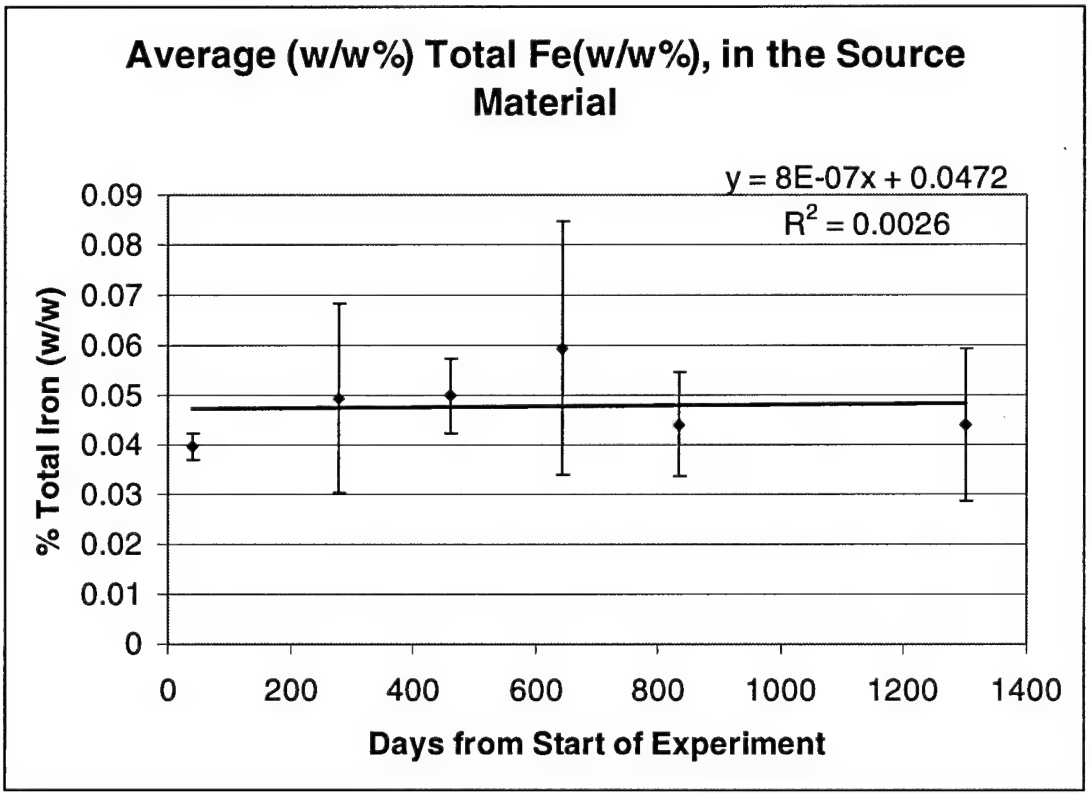


**Table 3: Average of Total Iron in the Source Material over Time**

| Date | Days Since start of Experiment | Average (w/w%) | Standard Deviation |
|---|---|---|---|
| Jan-96 | 40 | 0.044 | 0.015 |
| Sep-96 | 279 | 0.044 | 0.010 |
| Mar-97 | 462 | 0.059 | 0.025 |
| Sep-97 | 644 | 0.050 | 0.008 |
| Mar-98 | 835 | 0.049 | 0.019 |
| Apr-99 | 1300 | 0.040 | 0.003 |

**Figure 21. Average (n=4) Total Iron (w/w%) of Source Material**



### *8.4 Discussion*

Analyses from the field study clearly showed that the aquifer is heterogeneous in iron content. This heterogeneity was expected, since larger spatial variabilities over the site region for organic carbon content and hydrocarbon sorption coefficients had been reported by MacIntyre et al. 1998. The only consistent decreasing trends in total, amorphous and Fe (II) in site aquifer material were observed in the near field, primarily in samples closest to the source emplacement (N-4 and N-5). This was expected from the solution chemistry, as these were the areas (0-10 meters from the source) that had the highest dissolved hydrocarbon concentrations through time (Appendix, Table A1). The methane concentration also suggested that this was an area where anoxic degradation was taking place. In addition, the samples from section N-5 after initial emplacement of the source material were approximately two to three times higher in iron (II) than the rest of the aquifer (there are no background values for this location). This section corresponded to the highest outflow from the source material and suggests that the majority of the iron reduction may have occurred within (or very near) the source material, with the Fe (II) then deposited on the nearby sediments. This increase in sediment-bound Fe (II) also demonstrates that Fe (II) upgradient may sorb to the sediments, so dissolved Fe (II) concentrations are only a non-conservative indicator of microbial oxidation by iron (III) oxides in the aquifer. Therefore, the remaining capacity of an aquifer to degrade hydrocarbons through reduction of Fe (III) oxides cannot be calculated

by simple mass balance using the initial Fe (III) mineral content and loss Fe (III) minerals as measured by aqueous Fe (II) concentrations.

# 9. Laboratory experiments with aquifer material

These laboratory experiments were conducted to explore reactions of site aquifer materials and iron-containing minerals with hydrocarbons in controlled environments. The first experiment set, "Batch Microbial Experiments," was designed to give possible indications of what processes might be observed in the NATS field situation. The second experiment, "Magnetite Experiment," was designed to examine formation of secondary minerals (e.g. magnetite) during the consumption of hydrocarbons as a function of dissolved Fe (II) concentration.

## *9.1. Batch Microbial Experiments*

### 9.1.1. Experimental Design and Sampling Methods

These experiments were designed to provide optimal conditions for microbial oxidation of organics by iron oxides in the aquifer material so that resulting changes in the iron phases could be clearly identified. Three experiments were conducted as summarized in Table 4. The first was designed to assess whether a known iron-reducing microbe (*Geobacter metallireducens, also referred to as GS-15)* was capable of reducing the iron-bearing phases present in the CAFB aquifer. The second and third experiments examined reduction of iron-bearing phases by naturally-occurring bacteria, while using two different carbon sources, acetate and toluene.

The aquifer material used in each experiment was a freeze-dried and homogenized mixture of sediments taken above gradient from the field emplacement at the NATS site. Thus all treatments used a common sample of known mineralogy and surface area that allows for comparisons among the treatments. Autoclaved (120°C, 20 PSI, for 60 minutes) sediment was analyzed by XRD to determine if the mineralogy changed. As no noticeable change occurred, autoclaving was used to sterilize the sediment in each treatment and control. A NATS collaborator, Derek Lovely (University of Massachusetts, Amherst) supplied the *Geobacter metallireducens* culture used. The natural consortium of bacteria used here was gathered in September 1997. Sediment and pore water from an area in the aquifer with manifest ongoing microbial activity established by other NATS investigators was transferred by syringe into sealed, autoclaved serum vials containing a sterile acetate or toluene medium provided with an appropriate vitamin and mineral mixture. This encouraged growth of at least some of the natural consortium of bacteria. Table 5 shows the medium used for culturing GS-15 (Lovley, 1988) and the naturally occurring bacteria. The GS-15 was initially cultured in this medium with the addition of a hydrous iron oxide (HFO), of which a small amount transferred with the inoculate.

Experiment I used acetate medium and the Fe (III)-reducing microbe *G. metallireducens*. Experiment II used the naturally occurring consortium of bacteria as the Fe (III) reducer and the acetate medium as the electron donor. Experiment III also used the naturally occurring consortium of bacteria, but instead used a toluene medium; this medium was the same as the acetate medium except for the substitution of approximately 280 mg toluene per liter water for the acetate. Toluene is a component of the hydrocarbon mixture emplaced in the field experiment. *G. metallireducens* has been successfully grown on toluene, and it was anticipated that the naturally occurring bacteria would also use toluene as an electron-donor. Fe (II) production and, where appropriate, acetate loss, were analyzed as indicators of bacterial growth.

For all three experiments, ~15 g of the sediment mixture, appropriate food source/water mixtures and a vitamin/mineral supplement (totaling ~80ml) were combined in 100 ml serum vials fitted with a rubber septum, keeping a head-space of 80% $N_2$/20% $CO_2$. The mixtures were then autoclaved at 120 °C and 20 PSI for 60 minutes to sterilize the sediment, inoculated with the appropriate microbes (1ml of solution), and incubated at 30°C. All experiments were conducted in triplicate, except for the media only vials, which were duplicates. Analysis results for these samples include averages and Coefficient of Variation, expressed as a percentage (CV%), except the media vials, which were below the detection limit in Fe (II) and Total Fe Analyses.

Three different controls were studied. Abiotic (uninoculated) controls containing the sediment mixture, the appropriate medium and vitamin/mineral mixtures were used to assess the extent of iron reduction in the absence of microbial activity. Controls without sediments, but with the medium, vitamin/mineral mixtures and inoculate were used to determine the amount of iron unavoidably transferred along with the inoculum. Controls containing only the media and vitamin/mineral mixture were analyzed to establish baseline concentrations of the organics and Fe. The experimental treatments were allowed to incubate at 30°C for six months. After the incubation period, the samples were opened, filtered through a 0.45 μm cellulose filter to separate the solids, for characterization by a suite of methods, and the supernatant, for Fe (II) and acetate analysis. The filtered solids were rinsed twice with small aliquots of deionized water and freeze dried for iron extraction.

After the experiment, there was concern that the sediments were not completely sterilized and that naturally occurring microbes grew in all sediment containing vials. Specifically, the abiotic controls had considerable generation of Fe (see Tables 6 and 7). Therefore, an additional set of vials, labeled sediment control, were prepared that included the sediment mixture, growth media with the vitamin and mineral mixture. These vials were autoclaved like the three above treatments, but were allowed to incubate at 30°C for only three days to minimize any microbial growth; this three-day abiotic control is referred to as the "control" whereas the 6-month "abiotic" treatment is now referred to as "sediments only" since it appears that it was not truly an abiotic system.

**Table 4: Experiments investigating microbial oxidation of organics by Aquifer material**

| Experiment | Substrate | Microbes Used | Growth Medium |
|---|---|---|---|
| I | CAFB aquifer material | *Geobacter metallireducens* | Acetate |
| II | CAFB aquifer material | Natural consortium from CAFB | Acetate |
| III | CAFB aquifer material | Natural consortium from CAFB | Toluene |

**Table 5: Acetate Medium for GS-15 and Naturally Occurring Bacteria**

| Constituents | g/L deionized water |
|---|---|
| $NaHCO_3$ | 2.5 |
| KCl | 0.1 |
| $NH_4Cl$ | 0.5 |
| $NaH_2PO_4\ H_2O$ | 0.6 |
| $NaCH_3COO$ | 2.7 |

**Table 6: Amount of Total Fe (mg/L) in Solution**

(See text for a description of the samples)

| Naturally Occurring | | Average Fe (ppm) | %CV |
|---|---|---|---|
| **Acetate Vials** | Seds. Only | 11.57 | 20.20 |
| | Seds.+ Microbes | 11.32 | 8.67 |
| | Microbes Only | 1.00 | 11.89 |
| | Media Only | <0.05 | |
| **Toluene Vials** | Seds. Only | 12.19 | 16.07 |
| | Seds.+ Microbes | 10.55 | 8.87 |
| | Microbes Only | 0.98 | 3.33 |
| | Media Only | <0.05 | |
| **GS-15 Innoculum** | | | |
| **Acetate Vials** | Seds. Only | 12.59 | 56.10 |
| | Seds.+ Microbes | 12.90 | 9.95 |
| | Microbes Only | 8.09 | 8.60 |
| | Media Only | <0.05 | |
| | Abiotic Control | <0.05 | |

**Table 7: Concentration of Fe (II) (ppm) in Solution**

| Naturally Occurring | | Average Fe (ppm) | %CV |
|---|---|---|---|
| **Acetate Vials** | Seds. Only | 3.92 | 17.35 |
| | Seds.+ Microbes | 3.80 | 6.15 |
| | Microbes Only | 0.38 | 15.21 |
| | Media Only | <0.05 | |
| **Toluene Vials** | | | |
| | Seds. Only | 4.16 | 13.02 |
| | Seds.+ Microbes | 4.21 | 31.48 |
| | Microbes Only | 0.40 | 16.21 |
| | Media Only | <0.05 | |
| | | | |
| **GS-15 Innoculum** | | | |
| **Acetate Vials** | Seds. Only | 3.54 | 37.96 |
| | Seds.+ Microbes | 2.93 | 74.63 |
| | Microbes Only | 2.39 | 10.64 |
| | Media Only | <0.05 | 5.04 |
| | | | |
| | Abiotic Control | <0.05 | |

### 9.1.2. Results

Total iron in solution (Table 6) was similar in all three experiments for all vials containing sediments, and ranged from 10.6-12.9 ppm. When looking at the coefficient of variation for each sample, this variability was similar in all treatments. The detection limit (0.05ppm) was determined by the lowest standard, this is well below the sample values. In the vials with only naturally occurring bacteria and no sediments, the total iron concentration was ≈1 ppm iron. However, in the vials with the GS-15 bacteria, the total iron concentration was considerably higher (8 ppm total iron); this was due to the amount of iron transferred with the inocula. All vials with only media and the three-day abiotic control ("Sed. Only") had iron concentrations below the detection limit of 0.05 ppm.

The amount of reduced iron in solution is given in Table 7. In the vials with sediments only, the Fe (II) concentration ranged from 3.5 to 4.2 ppm. The detection limit (0.05 ppm) was determined by the lowest standard, and was well below the sample values. As with the total iron, all vials containing sediments, with or without microbes, had similar Fe (II) concentrations, ranging from 2.9 to 4.2 ppm. One of the triplicates, GS-15 Inoculated, Sediments + Microbes (see Appendix, Table A7), had an anomalously low value, which, if removed, gives an average of 4.16ppm (SMD = 0.49). When taking this value, the range for all sediment containing samples is 3.5 to 4.2; again not significantly different when considering the CV%. These Fe (II) concentrations constitute ≈ 32-34% of the

total Fe for the systems: natural-consortium acetate medium, natural consortium toluene medium, and GS-15 acetate medium. The media vials inoculated with the GS-15 had Fe (II) concentrations of 2.4 ppm, whereas the media vials with the naturally-occurring bacteria inoculum had Fe (II) concentrations of approximately 0.4 ppm. Although the total Fe (II) was higher for the GS-15 microbes only samples, the percent Fe (II) of the total was lower (about 30%, vs. 40% for the natural consortium microbes-only samples, both media), likely reflecting the higher initial Fe (III) content of the GS-15 vials. The vials containing just the media in all three treatments had concentrations below the detection limit of 0.05 ppm Fe (II), which suggests that most of the Fe was present in its reduced form in these treatments, and that background concentrations of Fe (II) were negligible compared to that produced by microbial activity. The sediment control vials that were incubated for only 3 days had no detectable Fe (II) in solution (i.e. < 0.05 ppm Fe (II)).

The average acetate consumed for the three replicates shown in Table 8 was determined for both the natural consortium and GS-15 treatments. The detection limit (23 mg/L), determined by three times the standard deviation of the blanks, was well below the sample values. Note that a similar determination of toluene consumed by the natural consortium in Experiment II cannot be made because of analytical difficulties due to toluene vaporization (see Methods in Chapter 7). Acetate consumed was calculated by subtracting the acetate concentration in the reacted vials from the acetate concentration in the media, and multiplying by the volume of media in each vial. For experiment III (GS-15 in

acetate), acetate consumption was greatest in the presence of both sediments and microbes, lower in the vials with sediments only, and lowest in the "microbes only" vials. Note, however, that because of the high variability (%CV), acetate consumption for the "sediments only" vials cannot be distinguished from either the "seds + microbes" or "microbes only" vials. In contrast, for experiment I (natural consortium in acetate) more acetate was consumed in the sediment only vials than in the sediment and bacteria vials, with the lowest consumption again for the "microbes only" vials. Again, however, because of the high variability, the acetate consumption among all vials is not significantly different. Last, acetate consumption was higher overall in the vials with the naturally-occurring bacteria compared with the GS-15.

The amount of total iron from the 5N HCl extraction and the amorphous iron determined by the 1N HCl extraction for the reacted sediments are listed in Table 9. The detection limit ($9.8 \times 10^{-5}$ w/w%) for both extractions was determined by three times the standard deviation of the blanks, converted to a w/w% using the average weight of a sample (0.25g) and the volume of the extract (0.02L); this is well below the sample values. There was negligible difference between the three experiments or between these and the abiotic control. In each treatment, the total iron was about 3 ppm and the amorphous iron was approximately 1 ppm. These results indicate that the majority of the sediment iron phases were not utilized by the bacteria over the 6 month incubation period, despite the high aqueous concentrations of iron. Note however, that if all sediment iron was released, the aqueous iron concentrations

would be much higher. A rough mass balance calculation to estimate amount of iron that would be released if ALL iron was solubilized is:

$$\frac{\left(\frac{15g\ sed}{vial}\right)*\left(\frac{3.4mg\ Fe}{g\ sed}\right)*\left(\frac{1000ml}{1L}\right)}{\left(\frac{80ml\ solution}{vial}\right)} = 637.5mg/L\,Fe$$

Only ~2% of the total iron available in the sediment is released into solution.

The surface iron concentrations of the reacted sediments determined by EDS are listed in Table 10. As discussed in Section (8.3), detection limits could not be determined for these analyses. In each of the three experiments, surface iron concentrations (weight % Fe) normalized to silica concentrations appeared to increase with reaction of the sediments with the bacteria, suggesting readsorption of solubilized iron. However, as the aqueous iron concentrations were similar with or without added microbes, these results may also indicate a difference in the form of resorbed Fe whereby resorbed iron covers the surface in a manner that more effectively masks the underlying mineral surfaces in the presence of bacteria. Note, however, that the Fe/Si ratios were highly variable, and as such trends in their values were not significant. The high variability of individual measurements of Fe/Si is an indication of the inherent variability of the homogenized sediment, as also seen for the field experiment. The concentration and/or form of the iron oxides on individual sediment grains is highly variable despite attempts to minimize variability through use of a size-fractionated, homogenized sediment.

Specific surface areas (SSA) of the sediments in the different treatments are given in Table 11. Detection limits for this method could not be obtained. However, before each day's analyses, the operation of the surface area instrument was calibrated to a known kaolinite reference standard (16.8 ± 0.8 $m^2/g$). In both experiments using the naturally occurring bacteria, surface area increased with reaction of the sediments with the bacteria. Additionally, the SSA of the control is similar to the sediment-only vials of these two experiments. In the experiment inoculated with GS-15, SSA was higher than in the experiments with naturally occurring bacteria and *decreased* with reaction of the sediments with the bacteria.

**Table 8: Average Acetate Consumed (µg) (n=3)**

| Naturally Occurring Acetate Vials | | Average Acetate Consumed (ug) | CV% |
|---|---|---|---|
| | Seds. Only | 12920 | 18 |
| | Seds.+ Microbes | 10005 | 36 |
| | Microbes Only | 8751 | 24 |
| | | | |
| GS-15 Innoculum Acetate Vials | | | |
| | Seds. Only | 3619 | 66 |
| | Seds.+ Microbes | 8978 | 26 |
| | Microbes Only | 1743 | 90 |

**Table 9: Average Amount of Fe Extracted from Reacted Sediments (n=3)**

| Naturally Occurring Acetate Vials | | 1N Fe Extraction (mg Fe/g sediment) | CV% | 5N Fe Extraction (mg Fe/g sediment) | CV% |
|---|---|---|---|---|---|
| | Seds. Only | 0.0694 | 29.1 | 0.3176 | 11.3 |
| | Seds.+ Microbes | 0.0850 | 22.2 | 0.3436 | 10.2 |
| | | | | | |
| Toluene Vials | | | | | |
| | Seds. Only | 0.1109 | 17.6 | 0.3473 | 10.1 |
| | Seds.+ Microbes | 0.1189 | 20.3 | 0.3345 | 10.3 |
| | | | | | |
| GS-15 Innoculum | | | | | |
| Acetate Vials | | | | | |
| | Seds. Only | 0.1016 | 24.0 | 0.3194 | 11.9 |
| | Seds.+ Microbes | 0.0753 | 36.9 | 0.3099 | 12.2 |
| | | | | | |
| | control | 0.0570 | 5.3 | 0.3080 | 0.9 |

**Table 10: Surface Fe Concentration (wt %, normalized to Si wt%) of the Sediments Determined by EDS**

| Naturally Occurring | | Average wt.% Fe | CV% |
|---|---|---|---|
| Acetate Vials | Seds. Only | 0.78 | 77.8 |
| | Seds.+ Microbes | 0.88 | 119.9 |
| | | | |
| | | | |
| Toluene Vials | Seds. Only | 0.60 | 77.3 |
| | Seds.+ Microbes | 0.66 | 83.0 |
| | | | |
| | | | |
| GS-15 Innoculum | | | |
| Acetate Vials | Seds. Only | 0.68 | 100.9 |
| | Seds.+ Microbes | 1.22 | 205.3 |
| | | | |
| | Control | 0.87 | 83.0 |

**Table 11: Average (n=3) Specific Surface Area ($m^2/g$) of Reacted Sediments**

| Naturally Occurring | | Average ($m^2/g$) | CV% |
|---|---|---|---|
| Acetate Vials | Seds. Only | 1.30 | 63 |
| | Seds.+ Microbes | 2.52 | 33 |
| | | | |
| Toluene Vials | | | |
| | Seds. Only | 1.89 | 52 |
| | Seds.+ Microbes | 2.43 | 13 |
| | | | |
| GS-15 Innoculum | | | |
| Acetate Vials | | | |
| | Seds. Only | 3.69 | 34 |
| | Seds.+ Microbes | 2.85 | 53 |
| | | | |
| | Control | 1.46 | 17 |

### 9.1.3. Discussion

It is apparent for several reasons that autoclaving the sediments at 120°C was insufficient to kill off all the naturally occurring bacteria. The concentration of total iron and iron (II) in solution is very similar for the vials with sediments only and the vials with sediments and bacteria in all three experiments. And when compared to the iron concentrations in the abiotic control, which is below detection limit, there has definitely been a reaction with the sediments over the course of the 180-day experiments. Also, the acetate consumption for the naturally occurring bacteria experiments was quite high in the sediment only vials, higher than in the vials inoculated with the bacteria. So, over the six-month incubation period, the naturally occurring bacteria in the sediments that survived the autoclaving had time to establish a viable population.

The analysis of surface iron concentration normalized to silica concentrations appeared to show a slight increase (although, due to the high CV%, probably, insignificant increase) in iron coating the particles. This may suggest that solubilized iron had been redeposited on the surface of the particles, perhaps in a form different than for the original iron phases.

The amount of the extractable iron in Sed. only versus the Sed.+microbes did not differ significantly between experiments or from the abiotic control. The amount of iron released to aqueous solution was only a small fraction of the total available iron. So, it appeared that six months was not enough time to show a significant reduction in the iron phases coating the sediments in a laboratory

setting. However, this may in part be an artifact of the experimental design. In an aquifer system, any release iron is continually removed via groundwater transport whereas high levels of aqueous iron are achieved in a batch laboratory experiment. These high iron concentrations may suppress further microbial activity and also allow for readsorption of Fe that may "poison" the iron oxide surfaces for further microbial degradation.

The larger surface areas seen in the GS-15 experiment in comparison to the two experiments with naturally occurring bacteria might be due to the transfer of the HFO gel on which the GS-15 was cultured. Such material has a very high specific surface area, 600 $m^2/g$ for fresh precipitate (Dzombak and Morel, 1990), and so, even a small amount could affect the surface area measured. The presence of the high surface area HFO precludes the evaluation of trends in the surface area of this system.

In summary, there was considerable consumption of Fe (III) oxides in the CAFB sediment by both GS-15 and naturally-occurring bacteria. The high levels of iron released may have been partially resorbed. The effects of both the high Fe concentrations and this resorption are unknown, but may have caused a decrease in microbially-assisted dissolution of the iron oxides. The majority of the sediment iron oxides were not consumed during the six-month incubation periods. These results indicate that either the capacity for iron reduction has not been reached (and thus longer incubation times are needed) or that the initial capacity diminishes over time due to the build up of iron in the system. While such a build up of aqueous iron is not expected in the field because of

groundwater movement, a similar decrease in capacity may occur in the field as iron released due to microbial activity is transported down-field and subsequently resorbed onto the aquifer materials.

## *9.2. Magnetite Formation Experiment*

Magnetite has been observed in contaminated aquifers under Fe(III) reducing conditions and is considered a by-product of microbial iron reduction. Iron-reducing bacteria use ferric iron as a terminal electron acceptor in an overall reaction reducing solid Fe (III) oxides to aqueous Fe (II). Magnetite is formed by rearrangement and water expulsion when aqueous Fe (II) sorbs into amorphous or poorly crystalline Fe (III) oxides on the surface of aquifer material grains. If magnetite is formed only in the presence of iron-reducing microbes, then magnetite production may be an indicator of microbial degradation of hydrocarbons. This study was designed to test by negation of the hypothesis that magnetite formation is microbial. This negation was investigated by searching for experimental abiotic production of magnetite under similar culture conditions to those required for microbial production of magnetite.

### 9.2.1. Design and Sampling Method

Batch cultures of *Geobacter metallireducens* (GS-15) were grown under strict anaerobic condition using the same growth media, 5ml of hydrous iron oxide (HFO) gel, and method as outlined in section 9.1.1. The amount of HFO

transferred to the biotic vials with the inoculate is trivial compared to the semi-solid HFO in the vials. Two experiments were designed, one a batch group inoculated with 1 ml of supernatant from a vial known to contain GS-15 and the other left abiotic. Triplicate vials were set up to which Fe (II), as an aqueous solution of $FeCl_2$, was added in the concentrations of 50, 100, 200, 400 and 800 mg /L. Abiotic controls were incubated along with the microcosms containing bacteria for five months at 30°C.

After one month, 5 ml aliquots of the solution were removed by syringe filter and tested for Fe (II) and acetate concentrations. After 5 months, the vials were opened, and the samples were vacuum filtered through a 0.45µm cellulose membrane. The filtrate was sub-sampled for total iron, Fe (II) and acetate analyses following the sample methods used for the field experiment (see section 7.1).

The filtered iron oxide solids were then rinsed twice with a small amount of DDI water to minimize the amount of Fe (II) from the pore water, and freeze dried. The dried samples were then homogenized and sub-sampled for 1 N HCl extraction, XRD analysis, and SEM/EDS analysis (see section 7.1 for details).

An additional 5 – 10 g sample of each dried sample was placed on a plastic weighboat and swirled over a strong magnet to concentrate the magnetized particles. The magnetic fraction was subjected to XRD and SEM/EDS analysis as for the bulk sediment; prior to SEM/EDS, this fraction was examined under an optical microscope in order to initially identify the characteristics (size and morphology) of the magnetite. Magnetite could be

distinguished from associated HFO by its optical properties (e.g. degree of opaqueness and octahedral structure).

### 9.2.2. Results

Average acetate concentrations in the biotic vials after 5 months was significantly less than in the abiotic vials, except in the 50ppm and 800 ppm Fe (II) additions (Table 12). The detection limit (93 mg/L), determined by the lowest standard, was well below the sample values. These results show that there was degradation of the acetate, most likely due to microbial activity (Table 12). Note, however, that there appears to be a loss of acetate in all vials (≈ 13-30% for the abiotic vials), most likely due to an unknown amount of hydration of the ammonium acetate. The original stock compound was not new and appeared to be quiet wet. The presumed initial concentration of acetate added as media was 2.7 mg/L $NaCH_3COO$, which is equivalent to 1.9 g/L $CH_3COO^+$. Furthermore, the acetate loss in abiotic vials was variable, with greater loss in the 50 and 800 ppm (lowest and highest Fe (II) additions). The difference between the abiotic and biotic vials was generally greater at lower Fe (II) additions, suggesting a possible effect of Fe (II) on microbial activity (Table 12).

**Table 12: Average Acetate Concentrations in Vials after 5 months**

Sample designations refer to the amount of Fe (II) added in mg/L. The controls were vials with no added Fe (II) for each of the abiotic and biotic systems.

| Amount of Added Fe (II) (µg/ml) in Vial | Average Acetate Conc. (mg/L) | n | Standard Mean Deviation |
|---|---|---|---|
| Biotic Samples | | | |
| 0 | 1390 | 2 | 96 |
| 50 | 1205 | 2 | 37 |
| 100 | 1447 | 3 | 82 |
| 200 | 1546 | 2 | 64 |
| 400 | 1463 | 3 | 101 |
| 800 | 1671 | 3 | 65 |
| Abiotic Samples | | | |
| 0 | 1681 | 2 | 116 |
| 50 | 2018 | 3 | 617 |
| 100 | 1661 | 3 | 103 |
| 200 | 1841 | 3 | 27 |
| 400 | 1716 | 3 | 143 |
| 800 | 1542 | 2 | 111 |

#### 9.2.2.1. Microscopic Observation of Magnetite

The optical microscope used was not fitted with a camera, and descriptions of the visual observations are reported here. Particles size estimates were based on distance calibrated graduated crosshairs on the optical microscope. In general, the magnetite particles were larger in the biotic samples than in the abiotic samples. Examination of the magnetic separates by SEM also clearly shows differences between magnetite formed in the abiotic samples versus the biotic samples (see Figures 22-25). Magnetite is clearly discernable by its cubic morphology and by its high iron content. Specifically, the biotic samples contained larger magnetite crystals, typically 10-20 μm. Abiotic samples had magnetite crystals typically 0.5-1μm, one tenth the average size seen for the biotic samples. The magnetite particles seen in the biotic samples appear to be distinct crystals, not associated with any other substrate. The magnetite particles that formed in the abiotic samples appear to be associated with the HFO matrix and do not appear as distinct particles.

**Figure 22: Magnetite Crystals Formed in Abiotic Vials with Addition of 800 ppm Fe (II)**

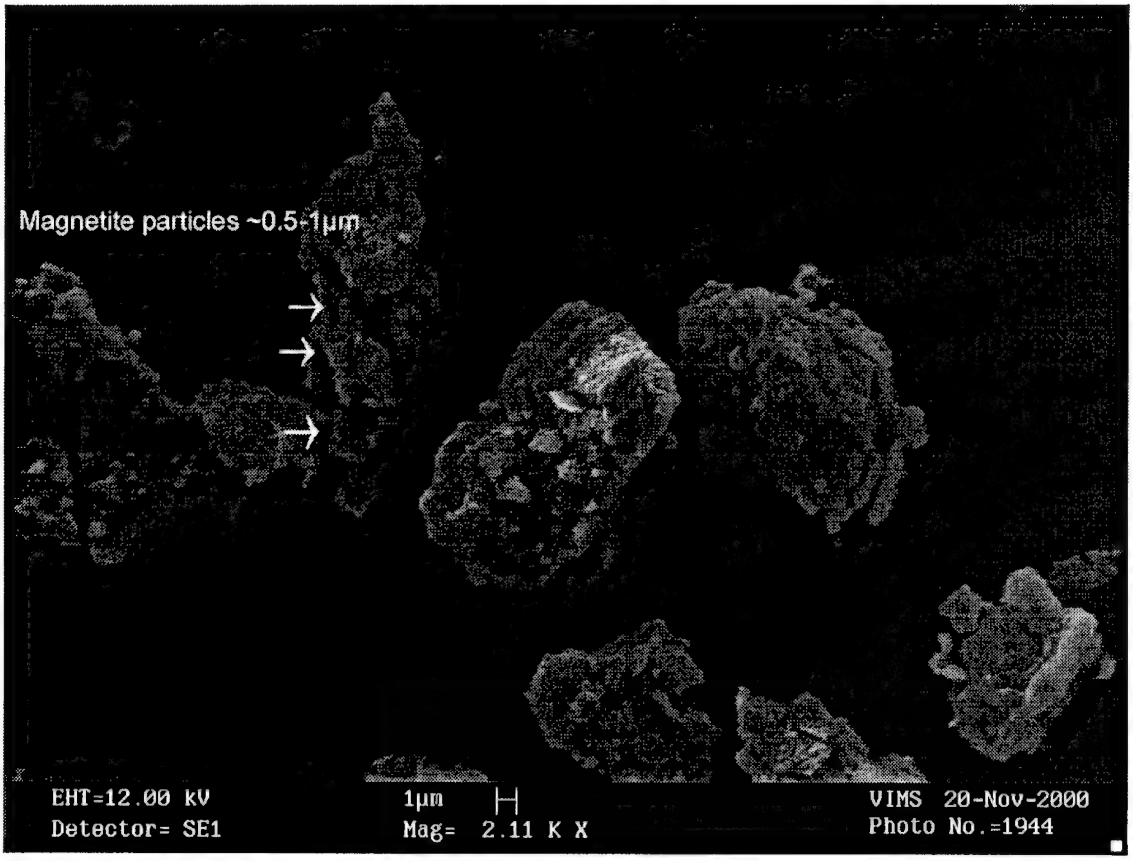


**Figure 23: Magnetite Crystals Formed in Abiotic Vials with Addition of 800 ppm Fe (II)**

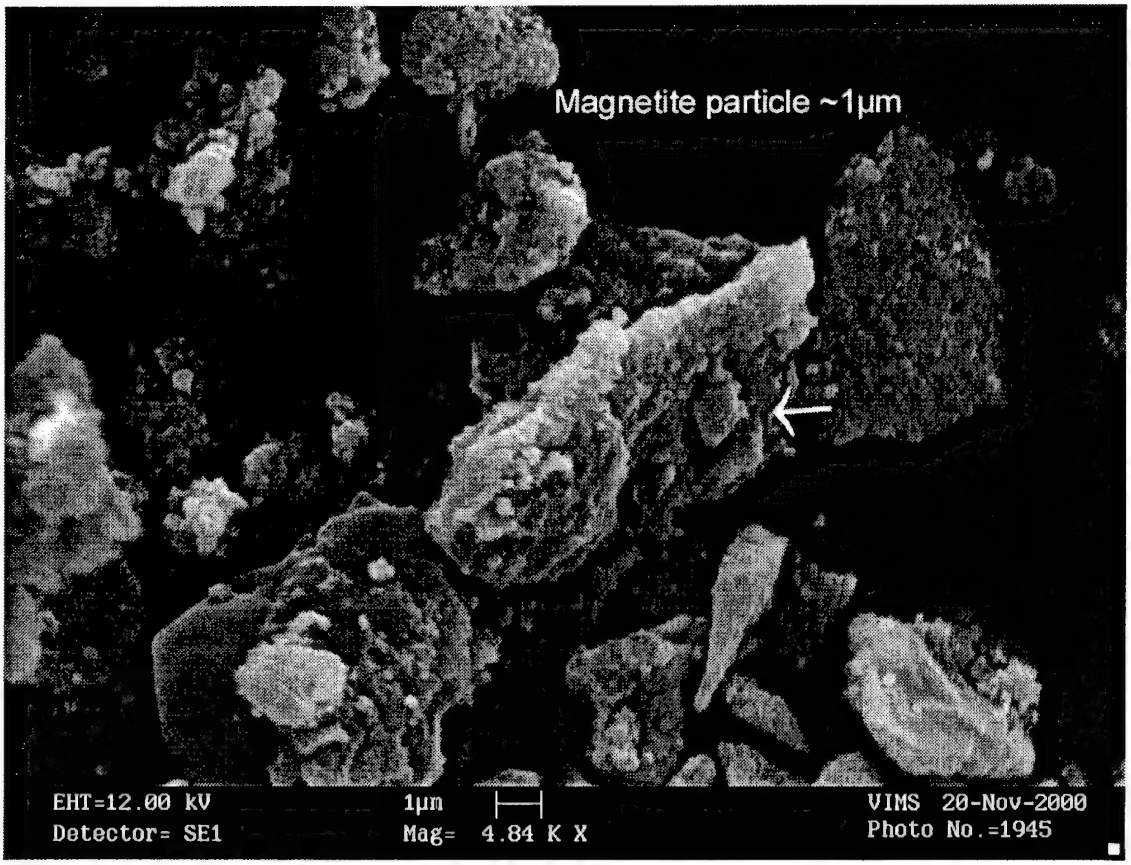


**Figure 24: Magnetite Crystals Formed in Biotic Control Vial**

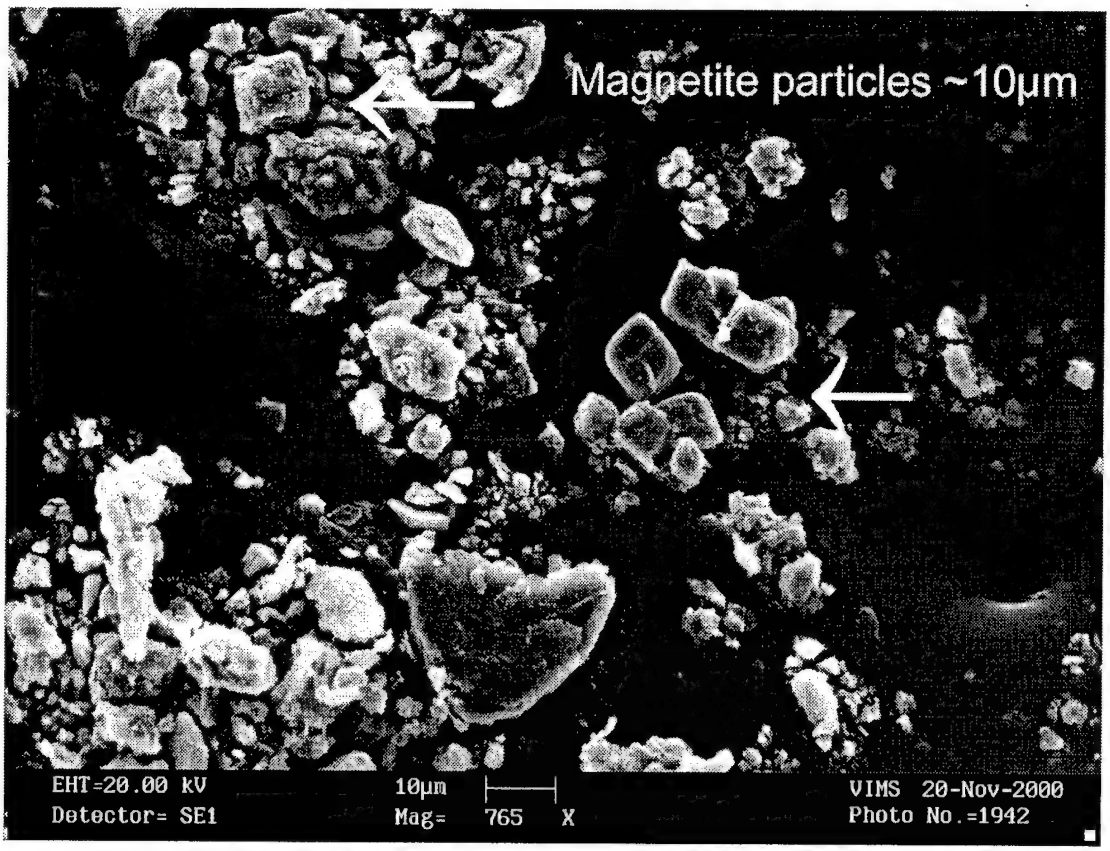
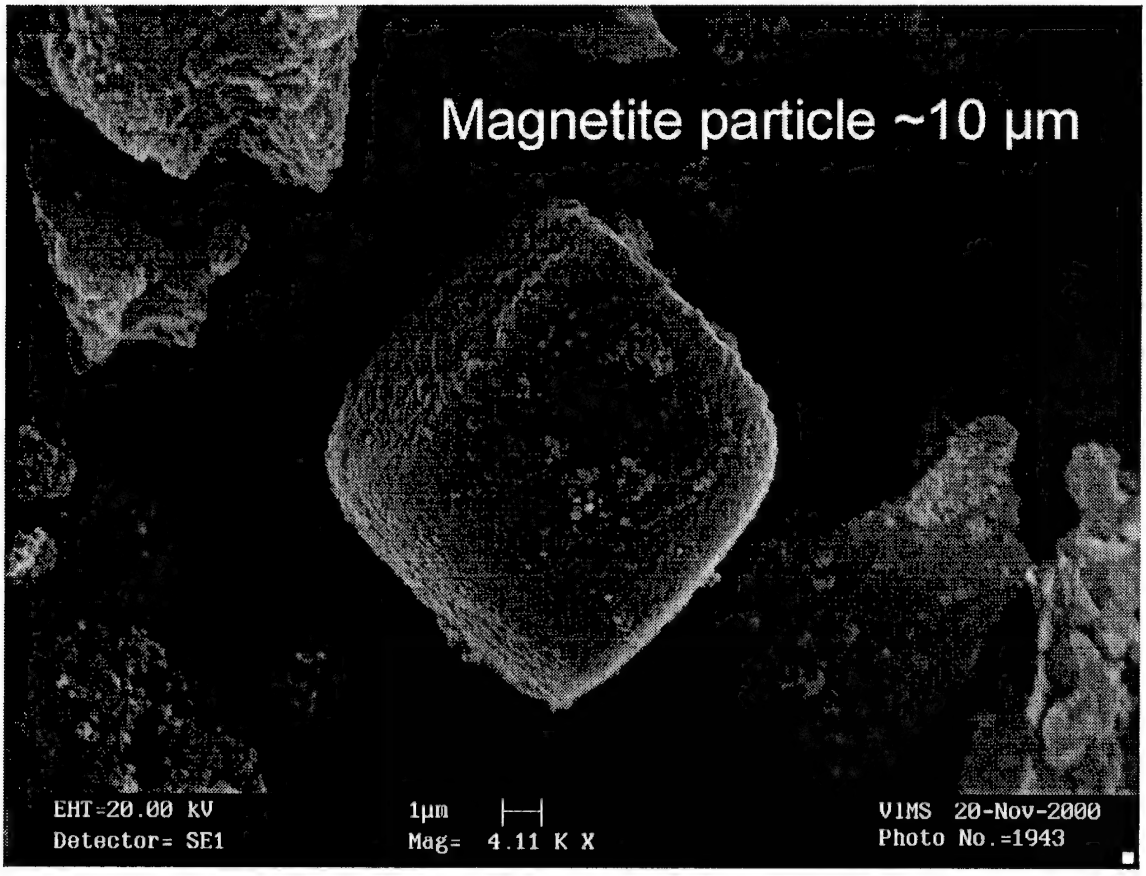


**Figure 25: Single Magnetite Crystal Formed in Biotic Control Vial**



### 9.2.2.2. [Fe (II)] Variation with Time

Table 13 shows the concentration of Fe (II) in solution after 1 month and 5 months. The detection limit (0.05 mg/L) was determined by the lowest standard, this is well below the sample values. After 1 month, the Fe (II) concentrations in the abiotic vials were all lower than those initially introduced. This indicated that some Fe (II) in the abiotic vials had sorbed into the hydrated iron gel. Fe (II) production in biotic vials kept the ferrous iron concentrations higher than in the comparable abiotic vials. However, after one month, Fe (II) concentrations were lower than the initial values in the vials with ≥ 200 mg/L initial Fe (II), again suggesting some resorption into the gel. After 5 months, there was little Fe (II) remaining in solution in either the abiotic or biotic vials, with moderate levels only for the highest initial Fe (II) 800 mg/L vials. This suggested that most of the Fe (II) was incorporated into the HFO or other iron phases in both biotic and abiotic vials. There are several stoichiometric relationships between the amount of acetate consumed to the amount of Fe (II) and magnetite produced (Lovely and Phillips, 1988).

$CH_3COO^- + 8\ Fe^{3+} + 3H_20 \rightarrow 8Fe^{2+}\ HCO^{3-} + CO_2 + 8H^+$ $\Delta G° = -814$

$CH_3COO^- + 24\ Fe(OH)_3 \rightarrow 8Fe_3O_4 + HCO^{3-} + CO_2 + 37H_2O$ $\Delta G° = -712$

$Fe^{2+} + 2Fe(OH)_3 \rightarrow Fe_3O_4 + 2H_20 + 2H^+$ $\Delta G° = -90$

However, since the concentration of magnetite formed was not determined, no relationship between the aquaeous concentration of Fe (II) and acetate can be determined.

**Table 13: Fe (II) Concentration (mg/L) in Vials after 1 and 5 Months**

| Amount of Fe(II) Added | Average Fe(II) (ppm) After 1 Month | | Sample Mean Deviation | Average Fe(II) (ppm) After 5 Months | | Sample Mean Deviation |
|---|---|---|---|---|---|---|
| Biotic Samples | | n | | | n | |
| 0 | 123.88 | 2 | 4.37 | 16.36 | 2 | 58.64 |
| 50 | 204.16 | 2 | 43.53 | n/a | 2 | |
| 100 | 138.90 | 3 | 47.73 | 15.17 | 3 | 0.58 |
| 200 | 179.44 | 2 | 20.56 | 15.53 | 2 | 0.47 |
| 400 | 310.09 | 3 | 39.18 | 14.46 | 3 | 2.45 |
| 800 | 739.75 | 3 | 38.11 | 91.25 | 3 | 3.43 |
| | | | | | | |
| Abiotic Samples | | | | | | |
| 0 | 0.12 | 2 | 0.37 | 0.24 | 2 | 0.02 |
| 50 | 5.77 | 3 | 1.00 | 3.57 | 3 | 0.89 |
| 100 | 19.94 | 3 | 0.86 | 6.51 | 1 | n/a |
| 200 | 41.08 | 3 | 0.91 | 13.42 | 3 | 3.60 |
| 400 | 110.91 | 3 | 14.10 | 36.59 | 1 | n/a |
| 800 | 663.34 | 2 | 102.36 | 87.68 | 1 | n/a |

### 9.2.2.3.XRD Data

The mineralogy of the abiotic and biotic systems was distinct after 5 months time. Magnetite formed in all biotic vials (Figure 26), whereas XRD diffraction patterns of the abiotic vials show no evidence of magnetite formation (Figure 27). Despite microscopic and magnetic evidence, the very small crystalline size and low abundance of magnetite may have made XRD analysis inconclusive for the presence of magnetite in the abiotic vials. Residual ferrihydrite (amorphous hydrated ferric oxide) is present in all abiotic and biotic vials; as evidenced by the large "blob-like" peak in the 40-48 2-Θ range. The biotic vials to which ≤100 ppm Fe (II) was added contain no goethite (ferric oxyhydroxide, α-FeOOH); otherwise, goethite formation increases with increasing Fe (II) addition. Goethite formed in all abiotic vials except the controls. Siderite (ferrous carbonate, $FeCO_3$) formed in all biotic vials, except the 800 ppm Fe (II) additions; the greatest abundance of siderite was in the 200 ppm Fe (II) additions. Notably, this reduced iron phase, siderite was not detected in any abiotic vial. Siderite would not be expected to be present in the field study because the low pH (about 5) of the groundwater would dissolve the mineral. Hematite (ferric oxide, $\alpha$-$Fe_2O_3$) formed in vials with 200 or 400 ppm Fe (II) additions for the biotic systems and all abiotic vials except the 800 ppm Fe (II) additions. HFO is a thermodynamically unstable phase (Dzombak and Morel, 1990) and transforms to a more crystalline iron oxide such as goethite or hematite with aging.

**Figure 26: XRD Diffraction Patterns Of Solids in The Biotic System Magnetite Formation Experiment**

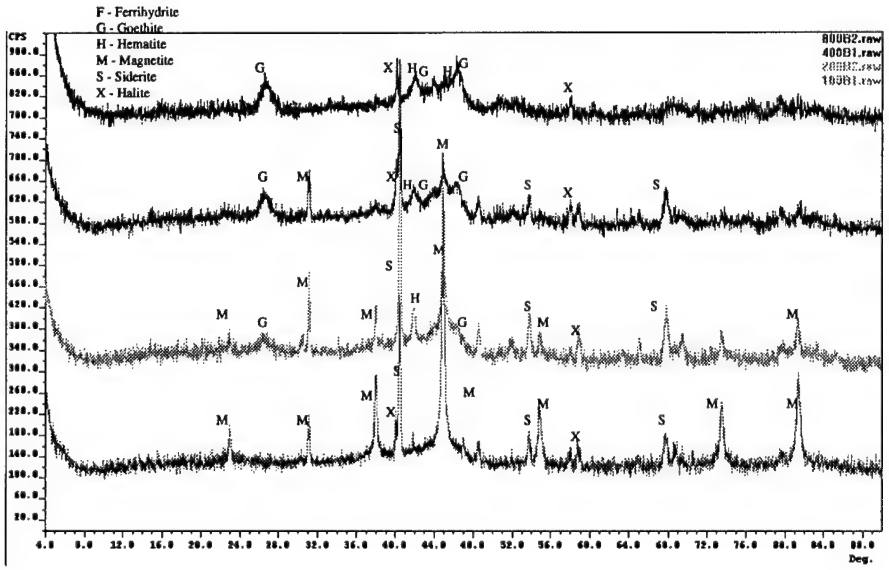
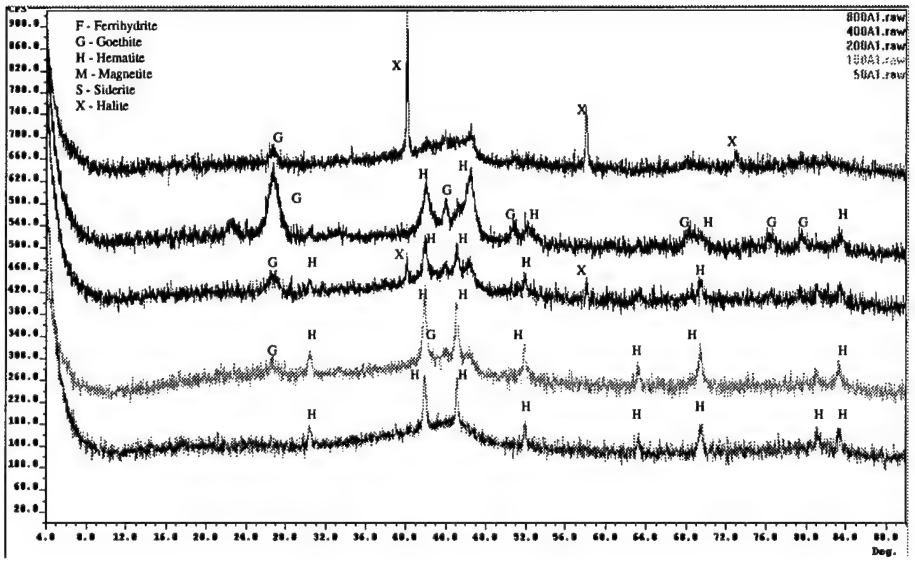


**Figure 27: XRD Diffraction Patterns Of Solids in the Abiotic System Magnetite Formation Experiment**



### 9.2.3. Discussion

As evidenced by magnetic affinity, optical microscopy and SEM/EDS, magnetite was formed in all the biotic vials and in the abiotic vials to which more than 200 ppm Fe (II) was added. After 1 month, the iron gel in each of the abiotic vials sorbed some of the Fe (II) in solution. There is no evidence for generation of Fe(II) in the abiotic vials (Fe(II) steadily decreases from the starting value), which indicates that these systems were indeed abiotic. There also was no evidence of microbes in the SEM images of the solids in the abiotic vials; however, these solids were *not* critical point dried and thus the SEM data are not conclusive that the system was abiotic. After 5 months almost all of the excess Fe (II) introduced into the vials, as well as the Fe (II) produced by the microbes, was removed from solutions either by sorption or precipitation. As sorption of Fe (II) into the iron gel is the first step of magnetite formation, the loss of aqueous Fe (II) suggests the possibility of production of micro-crystalline magnetite inside the ferrihydrite matrix. The small crystallite size of this first formed magnetite is not detectable by powder XRD. In abiotic systems, magnetite did not form to a size or abundance detectable by XRD, even after 5 months. In contrast, the magnetite crystals were larger in the biotic systems, clearly detectable by XRD. Nonetheless, formation of magnetite in the abiotic microcosms suggests that magnetite crystallization is a chemical process occurring independent of microbial activity, and magnetite production in itself

may not be a sufficient indicator of microbial degradation of hydrocarbons by ferric oxides.

More magnetite was formed in the biotic vials than in the abiotic. There is also a trend of decreasing magnetite production with increasing Fe (II) addition in the biotic systems, but increasing magnetite production with increasing Fe (II) addition in the abiotic systems. With increasing Fe (II) concentration, more Fe (II) is sorbed into the iron gel, forming a Fe (II)/Fe (III) complex that rearranges its matrix structure and expels water, forming magnetite. When the iron gel becomes pre-magnetite, it is in a more reduced state and may be less effective for microbial oxidation of acetate. So, one might expect the effects on magnetite formation observed here. Additionally, considerably more Fe(II) is available for formation of secondary minerals in the biotic systems due to its generation from microbially activity which may in part account for the greater formation of magnetite in the biotic systems.

Siderite, $Fe(II)CO_3$, formed in the biotic systems, with the exception of the 800 ppm Fe (II) addition. The oxidation of acetate by the microbes reduces Fe (III) to Fe (II), increasing the [Fe (II)], and releases $CO_2$ in the form of bicarbonate (the system is buffered such that the pH remains near pH 7). Once the solubility product of siderite, $K_{sp}=10^{-10.67}$, is exceeded, siderite may precipitate. This can be used as an indicator of biological activity in closed systems containing Fe (II). There appears to be a maximum siderite formation with the 200 ppm Fe (II) addition. One would expect there to be less siderite formation in vials when there is less Fe (II) present, and that siderite formation

would increase at higher Fe (II) concentrations. The decrease in siderite, and magnetite, in the 400 ppm Fe (II) additions and the absence of siderite formation in the 800 ppm Fe (II) addition again indicates that the excess Fe (II) sorbed in the ferrihydrite gel decreases microbial oxidation of acetate.

# 10. Summary and Conclusions

The determination that naturally occurring microbes use iron oxides associated with aquifer materials in the anaerobic degradation of hydrocarbons is inconclusive from this study. There are some indications that the natural consortium of bacteria at the NATS site can degrade hydrocarbons, but statistically significant reduction in the iron minerals could not be determined from this study. The general hypothesis*: Microbial use of Fe (III) minerals for oxidation of hydrocarbons will produce changes in the iron minerals of NATS site sediments,* could not be statistically shown, primarily due to the large variability associated with a heterogeneous, alluvially deposited aquifer and the small hydrocarbon source that was used.

Groundwater analysis results from the NATS collaborators provide strong evidence that microbes used Fe (III) minerals, namely the appearance of dissolved Fe (II) as well as the appearance of breakdown products of the organic contaminants. Changes in the aquifer material due to dissimilatory Fe (III) reduction by microbes could not be determined from the testing of the secondary hypotheses.

The reduction of Fe (III) solids to Fe (II) results in the solubilization of the Fe (II), and thus releases iron from the sediments to the moving groundwater. The first secondary hypothesis, *Total iron content of the near source sediments decreases with time,* could not be conclusively shown. Neither the total iron in the sediments nor the Fe/Si from EDS analysis showed a statistically significant

temporal decrease. However, there did appear to be a decrease in the XRD peaks attributed to Fe (III) phases at one site closest to the source material.

Aqueous chemistry results show that Fe (II) released by microbial degradation in or close to the source region does not travel with the bromide tracer. The second hypothesis: *Fe (II) released during Fe (III) reduction is re-deposited in downfield sediments, showing that Fe (II) is non-conservative*, also could not be statistically determined. There was no significant increase over time in amorphous iron at downfield locations based on amorphous iron extractions of the core samples.

Previous research has demonstrated the availability of amorphous Fe (III) phases for microbial use and the greater difficulty in utilizing more crystalline forms of Fe (III) oxides. This preferential utilization of amorphous iron was not detectable in the laboratory experiment. The third hypothesis, *Amorphous Fe (III) will be preferentially consumed during microbial degradation of organic contaminants,* could not statistically determined. Total and amorphous Fe (III) in the aquifer material used in the laboratory microbial experiments showed no significant changes over time.

Magnetite has been found in contaminated aquifers under reducing conditions and is considered a by-product of microbial iron reduction. If magnetite forms only in the presence of iron-reducing microbes, its production could be a useful indicator of microbial degradation of hydrocarbons. Another, somewhat separate, laboratory experiment (the magnetite experiment) was conducted to explore the character of the secondary Fe (II) phases formed

during oxidation of organics and to test the following hypothesis. *Secondary Fe (II)-bearing phases, in particular magnetite, form only by microbial processes, and do not form in abiotic systems.* Not only was this hypothesis not supported, it appears that magnetite does indeed form under abiotic conditions. The XRD analysis for magnetite did not find any magnetite, probably due to the small size of the crystals. Affinity to a strong magnet and the SEM examination of the HFO found magnetite in both biotic and abiotic vials, thought the biotic vials had larger, more distinct crystals of magnetite.

This research provides three important findings. First, at least some of the natural consortium of bacteria in an aquifer is capable of degrading hydrocarbons in a reducing environment in the presence of iron oxides. This was shown in laboratory batch experiments by the amount of acetate consumed and the increase in Fe (II) in solution. Second, there may be some reduction of iron oxide minerals in the field near the NATS source emplacement. There was a decrease in the total iron oxides, shown by XRD, at the one core site closest to the source, and an increase in dissolved Fe (II) near the source, and the HC metabolites and degradation products were present in the groundwater. Third, magnetite should not be used as a primary indicator of microbial Fe (III) reduction in the field, since the laboratory magnetite formation study showed that magnetite was produced both biotic and abiotic systems.

The primary difficulty of this research was finding significant changes in the iron oxides in the aquifer. The two main causes for this difficulty were the size of the hydrocarbon source and the sampling design, neither of which were

determined by this researcher. The amount of hydrocarbon used for the source was limited by regulatory agencies and was not large enough to drive a significant portion of the aquifer anoxic. A source at least ten times the magnitude of the one used in NATS would be necessary to approach conditions found at a spill site. The sampling design for aquifer cores was determined by our collaborators to obtain limited microbial data and not for statistical testing of changes in heterogeneous aquifer materials. A much better method for determining the heterogeneity of the material would have to be developed. Instead of just looking at iron concentrations and variability over the entire aquifer, the variability of the iron oxides should be determined at individual locations. For example, assuming similar conditions to the NATS sampling, repeated measurements in one location through time were taken at 1-meter intervals to limit the physical disturbance and the addition of oxygen to the aquifer. If, before the start of the experiment, the variability of iron oxides in the aquifer on a 1-meter length scale for several locations throughout the aquifer were known, changes in the iron over time could be compared to this variability instead of the variability of the entire aquifer, potentially increasing one's ability to see significant changes over time.

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## Relevant Experience

December 1995-present. School of Marine Science, College of William and Mary.

Thesis research: "Characterization of the Minerals and Iron-Containing Phases of the Aquifer Material at Columbus AFB." The aquifer characterization is a part of the USAF Natural Attenuation Study (NATS) under US Air Force Office of Scientific Research grant #95-NA-328 to study the extent that iron phases are utilized by naturally occurring, iron-reducing bacteria in the degradation of light petrochemicals. Trace level iron analysis includes mineral-phase selective extraction and species-specific analysis methods including, UV/VIS, FAA and ICP spectrophotometry as well as mineral-phase identification by powder XRD, optical mineralogy and SEM/EDS. This information will be used to refine fate and transport models that predict the degradation of contaminants at military and industrial sites.

June 1997- August 1997 Armstrong Laboratories, Tyndall AFB, Florida
Air Force Office of Scientific Research, Summer Graduate Research Program

Gained field and laboratory experience in contaminant geochemistry, including proper collection and handling techniques of water and soil samples. Refined selective extraction and species-specific analysis methods using UV/VIS and FAA spectrophotometry. Also, conducted independent research on the abiotic formation of magnetite in iron reducing cultures, showing that magnetite formation is not necessarily indicative of microbial activity.

May 1995-September 1995. School of Marine Science, College of William and Mary.

Conducted field investigations of zinc and iron mobility at a BASF industrial landfill site in Lackey, Virginia including well emplacement and groundwater and aquifer sampling.

September 1992 - May 1993. College Of William and Mary, Senior Thesis.

Independently conducted the chemical analysis of ground and surface waters from the closed College landfill . Installed and monitored piezometers around the landfill. Used EPA suggested methods for analysis of inorganic compounds (collected from site), including atomic absorption spectrophotometry.

## Education

M. S. School of Marine Science, College of William and Mary
Projected Graduation August 1998
Concentration: Aqueous Geochemistry
Thesis Title: "Characterization of Iron-Bearing Phases used by Naturally-Occurring Microbes in the Anaerobic Degradation of Hydrocarbons"

B. S. Environmental Science, College of William and Mary. Received May 1993.

Concentration: Groundwater Hydrogeology and Chemistry.
Thesis Title: "Inorganic Chemical Analysis of the Closed College of William and Mary Landfill, Williamsburg, Virginia"

## Awards, Publications, Presentations

D. C. Powell, E. L. Libelo, T. G. Stauffer, W. G. MacIntyre, and C. J. Chisholm-Brause(1997) "Abiotic Formation of Magnetite in Iron Reducing Cultures". In: *EOS* **78** F200. Presented at the American Geophysical Union national meeting, San Francisco, December, 1997. In preparation for journal publication.

Chisholm-Brause, C. J., D. C. Powell, and E. L. Libelo (1997) "Effect of anaerobic microbial degradation of organic contaminants on the iron geochemistry of aquifer sediments". *Invited*. Presented at the American Chemical Society national meeting, San Francisco, April, 1997.

October 1992, $500 Minor Research Grant from the College of William and Mary for Senior Thesis Project. The usual maximum for this grant is $300.

## Scientific Society Memberships

The Geochemical Society
American Geophysical Union

# 12. APPENDIX

## Table A1. Solution Chemistry of the NATS Groundwater Over Time

| Zone | Date | $Fe^{+2}$ (mg/L) | B (ppb) | T (ppb) | E (ppb) | X (ppb) | N (ppb) | DIC (mM) | CH4 (µM) | NO3- (ppm) | SO4-2 (ppm) | Br- (ppm) | Cl- (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| -10 - 0m | 24-Aug-95 | 0.42 | | | | | | 1.48 | | | | | |
| -10 - 0m | 27-Sep-95 | 0.41 | | | | | | 1.89 | 0.09 | | | | |
| -10 - 0m | 8-Dec-95 | 0.01 | | | | | | | | 4.56 | 1.56 | | 6.48 |
| -10 - 0m | 9-Jan-96 | 0.41 | | | | | | 1.99 | 0.23 | 1.34 | 2.39 | 0.04 | 4.55 |
| -10 - 0m | 15-Mar-96 | 0.17 | 0 | 0 | 0 | 0 | 0 | | | 1.86 | 2.05 | 0.04 | 3.80 |
| -10 - 0m | 29-Apr-96 | 0.28 | 0 | 0 | 0 | 0 | 0 | 1.95 | 0.23 | 5.26 | 2.02 | 0.05 | 3.44 |
| -10 - 0m | 4-Sep-96 | 0.75 | 0 | 0 | 0 | 0 | 0 | 2.02 | 0.28 | 1.96 | 3.14 | 0.05 | 4.58 |
| -10 - 0m | 6-Mar-97 | 0.34 | 0 | 0 | 0 | 0 | 0 | 1.98 | 0.11 | 1.69 | 3.39 | 0.01 | 3.28 |
| -10 - 0m | 4-Sep-97 | 0.45 | | | | | | 2.98 | 1.20 | 1.79 | 5.04 | 0.03 | 4.04 |
| -10 - 0m | 14-Mar-98 | 0.28 | | | | | | 2.32 | 0.30 | 2.14 | 6.74 | 0.06 | 2.74 |
| -10 - 0m | 22-Jun-99 | | 0 | 0 | 0 | 0 | 0 | | | | | | |
| | | | | | | | | | | | | | |
| 0-5m | 24-Aug-95 | 0.07 | | | | | | 1.42 | | | | | |
| 0-5m | 27-Sep-95 | 0.05 | | | | | | 1.70 | 0.41 | | | | |
| 0-5m | 9-Jan-96 | 0.09 | 0 | 4 | 22 | 11 | 15 | 1.83 | 1.37 | 4.69 | 2.46 | 0.36 | 7.03 |
| 0-5m | 15-Mar-96 | 0.03 | 3 | 0 | 11 | 8 | 0 | | | 3.67 | 1.78 | 0.33 | 5.06 |
| 0-5m | 29-Apr-96 | 0.62 | 50 | 355 | 473 | 203 | 240 | 1.98 | 7.99 | 1.85 | 1.07 | 3.46 | 5.10 |
| 0-5m | 4-Sep-96 | 1.35 | 142 | 1428 | 1616 | 621 | 1837 | 2.56 | 8.85 | 2.45 | 1.49 | 1.37 | 5.76 |
| 0-5m | 6-Mar-97 | 2.44 | 203 | 4320 | 1947 | 1533 | 1727 | 2.76 | 17.87 | 1.66 | 2.32 | 0.86 | 5.17 |
| 0-5m | 4-Sep-97 | 2.62 | 48 | 60 | 70 | 83 | 160 | 3.20 | 28.58 | 1.87 | 2.09 | 0.51 | 4.68 |
| 0-5m | 14-Mar-98 | 1.91 | 127 | 2018 | 1133 | 1020 | 934 | 3.58 | 50.33 | 1.67 | 2.56 | 0.52 | 3.67 |
| 0-5m | 22-Jun-99 | | 285 | 2474 | 2631 | 2315 | 1877 | | | | | | |
| | | | | | | | | | | | | | |
| 5-10m | 24-Aug-95 | 0.15 | | | | | | 1.58 | | | | | |
| 5-10m | 27-Sep-95 | 0.10 | | | | | | 1.71 | 0.18 | | | | |
| 5-10m | 8-Dec-95 | 0.02 | 17 | 490 | 156 | 167 | 5 | | | 5.10 | 1.04 | 0.32 | 6.16 |
| 5-10m | 9-Jan-96 | 0.20 | 0 | 13 | 29 | 34 | 23 | 1.92 | 0.29 | 5.81 | 1.78 | 0.43 | 7.67 |
| 5-10m | 15-Mar-96 | 0.37 | 12 | 187 | 134 | 108 | 0 | | | 5.17 | 1.53 | 0.69 | 6.49 |
| 5-10m | 29-Apr-96 | 0.65 | 40 | 373 | 401 | 325 | 155 | 2.20 | 1.84 | 1.80 | 2.79 | 1.97 | 4.84 |
| 5-10m | 4-Sep-96 | 3.18 | 50 | 169 | 635 | 223 | 1182 | 2.52 | 4.95 | 3.51 | 2.03 | 0.82 | 5.60 |
| 5-10m | 6-Mar-97 | 1.06 | 46 | 220 | 327 | 229 | 438 | 2.25 | 5.47 | 2.75 | 3.05 | 0.34 | 4.88 |
| 5-10m | 4-Sep-97 | 1.18 | 43 | 85 | 95 | 104 | 267 | 2.47 | 8.11 | 2.58 | 2.52 | 0.39 | 5.02 |
| 5-10m | 14-Mar-98 | 0.81 | 22 | 71 | 161 | 134 | 176 | 2.40 | 8.69 | 2.96 | 3.06 | 0.22 | 4.18 |
| 5-10m | 22-Jun-99 | | 72 | 29 | 478 | 568 | 395 | | | | | | |
| | | | | | | | | | | | | | |
| 10-15m | 24-Aug-95 | 0.39 | | | | | | 1.43 | | | | | |
| 10-15m | 27-Sep-95 | 0.34 | | | | | | 1.69 | 0.22 | | | | |
| 10-15m | 8-Dec-95 | 0.94 | 4 | 5 | 3 | 4 | 0 | | | 4.10 | 3.15 | 0.15 | 6.72 |
| 10-15m | 9-Jan-96 | 0.36 | 0 | 0 | 0 | 0 | 0 | 1.87 | 0.44 | 5.82 | 1.94 | 0.25 | 7.42 |
| 10-15m | 15-Mar-96 | 0.11 | 2 | 0 | 0 | 0 | 0 | | | 7.22 | 1.95 | 0.07 | 6.94 |
| 10-15m | 29-Apr-96 | 0.66 | 1 | 0 | 5 | 4 | 5 | 1.89 | 0.86 | 1.99 | 5.61 | 0.22 | 4.92 |
| 10-15m | 4-Sep-96 | 2.27 | 2 | 0 | 5 | 2 | 21 | 1.99 | 5.73 | 4.70 | 1.65 | 0.25 | 7.88 |
| 10-15m | 6-Mar-97 | 0.58 | 3 | 0 | 0 | 1 | 10 | 1.74 | 5.97 | 4.52 | 1.25 | 0.11 | 4.80 |
| 10-15m | 4-Sep-97 | 0.55 | 3 | 0 | 0 | 0 | 9 | 1.95 | 1.34 | 4.08 | 2.04 | 0.12 | 5.13 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10-15m | 14-Mar-98 | 0.44 | 2 | 0 | 0 | 1 | 1 | 2.02 | 1.07 | 4.20 | 2.10 | 0.11 | 4.74 |
| 10-15m | 22-Jun-99 | | 0 | 0 | 0 | 0 | 0 | | | | | | |
| | | | | | | | | | | | | | |
| 15-20m | 24-Aug-95 | 0.01 | | | | | | 1.54 | | | | | |
| 15-20m | 27-Sep-95 | 0.02 | | | | | | 1.62 | 0.05 | | | | |
| 15-20m | 8-Dec-95 | 0.02 | 3 | 0 | 0 | 0 | 0 | | | 4.88 | 2.33 | 0.06 | 6.05 |
| 15-20m | 9-Jan-96 | 0.19 | 0 | 0 | 0 | 0 | 0 | 1.71 | 0.17 | 5.50 | 1.65 | 0.11 | 6.57 |
| 15-20m | 15-Mar-96 | 0.33 | 0 | 0 | 0 | 0 | 0 | | | 6.13 | 1.32 | 0.08 | 5.77 |
| 15-20m | 29-Apr-96 | 0.42 | 0 | 0 | 0 | 0 | 0 | 1.82 | 0.69 | 2.18 | 4.17 | 0.05 | 4.00 |
| 15-20m | 4-Sep-96 | 1.88 | 0 | 0 | 0 | 0 | 0 | 2.03 | 1.04 | 5.64 | 1.62 | 0.08 | 6.63 |
| 15-20m | 6-Mar-97 | 0.35 | 0 | 0 | 0 | 0 | 0 | 1.92 | 0.90 | 4.30 | 1.18 | 0.03 | 4.10 |
| 15-20m | 4-Sep-97 | 0.37 | 0 | 0 | 0 | 0 | 0 | 1.91 | 0.54 | 5.29 | 2.81 | 0.04 | 5.80 |
| 15-20m | 14-Mar-98 | 0.23 | 0 | 0 | 0 | 0 | 0 | 1.94 | 0.23 | 6.54 | 2.44 | 0.04 | 5.76 |
| 15-20m | 22-Jun-99 | | 0 | 0 | 0 | 0 | 0 | | | | | | |
| | | | | | | | | | | | | | |
| 20-25m | 24-Aug-95 | 0.00 | | | | | | 1.49 | | | | | |
| 20-25m | 27-Sep-95 | 0.01 | | | | | | 1.67 | 0.04 | | | | |
| 20-25m | 9-Jan-96 | 0.00 | 0 | 0 | 0 | 0 | 0 | 1.88 | 0.08 | 4.77 | 1.20 | 0.06 | 5.93 |
| 20-25m | 15-Mar-96 | 0.09 | 0 | 0 | 0 | 0 | 0 | | | 3.80 | 1.15 | 0.05 | 3.83 |
| 20-25m | 29-Apr-96 | 0.05 | 0 | 0 | 0 | 0 | 0 | 1.65 | 0.08 | 1.79 | 4.80 | 0.07 | 3.68 |
| 20-25m | 4-Sep-96 | 0.40 | | | | | | 1.88 | 0.07 | 5.13 | 1.37 | 0.08 | 5.23 |
| 20-25m | 6-Mar-97 | 0.00 | | | | | | 1.71 | 0.12 | 4.76 | 1.10 | 0.03 | 4.14 |
| 20-25m | 4-Sep-97 | 0.00 | 0 | 0 | 0 | 0 | 0 | 1.86 | 0.04 | 5.92 | 2.07 | 0.04 | 5.19 |
| 20-25m | 14-Mar-98 | 0.00 | 0 | 0 | 0 | 0 | 0 | 1.82 | 0.04 | 7.04 | 2.07 | 0.05 | 5.44 |
| 20-25m | 22-Jun-99 | | 0 | 0 | 0 | 0 | 0 | | | | | | |
| | | | | | | | | | | | | | |
| 25-30m | 24-Aug-95 | 0.00 | | | | | | 1.78 | | | | | |
| 25-30m | 27-Sep-95 | 0.01 | | | | | | 1.72 | 0.04 | | | | |
| 25-30m | 9-Jan-96 | 0.09 | 0 | 0 | 0 | 0 | 0 | 1.99 | 0.11 | 5.25 | 1.87 | 0.03 | 5.36 |
| 25-30m | 15-Mar-96 | 0.01 | 0 | 0 | 0 | 0 | 0 | | | 5.59 | 1.33 | 0.03 | 4.32 |
| 25-30m | 29-Apr-96 | 0.02 | | | | | | 1.64 | 0.10 | 2.97 | 4.46 | 0.03 | 2.57 |
| 25-30m | 4-Sep-96 | 0.35 | | | | | | 1.85 | 0.06 | 5.54 | 0.60 | 0.05 | 5.29 |
| 25-30m | 6-Mar-97 | 0.00 | | | | | | 1.64 | 0.08 | 2.62 | 1.10 | 0.01 | 2.84 |
| 25-30m | 4-Sep-97 | 0.00 | | | | | | 1.76 | 0.12 | 5.99 | 2.30 | 0.05 | 5.54 |
| 25-30m | 14-Mar-98 | 0.00 | 0 | 0 | 0 | 0 | 0 | 1.96 | 0.06 | 5.74 | 2.71 | 0.04 | 4.59 |

**Figure A1: January 1996 Samples, Normal Plot of Amorphous Iron (1N HCl Extractable) Concentrations**

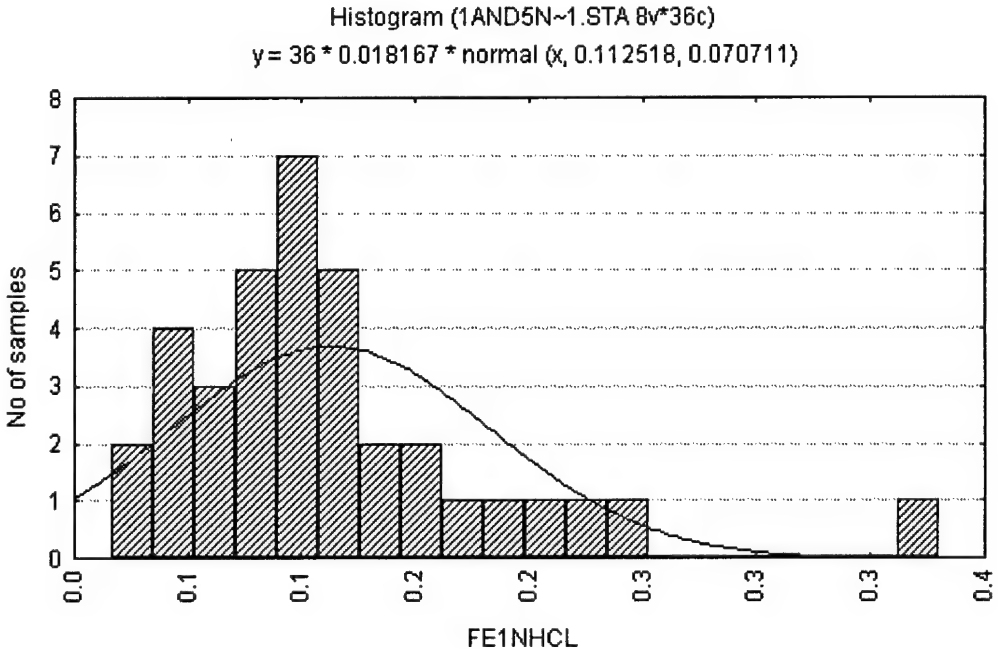


**Figure A2: January 1996 Samples, Log Normal Plot of Amorphous Iron (1N HCl Extractable) Concentrations**

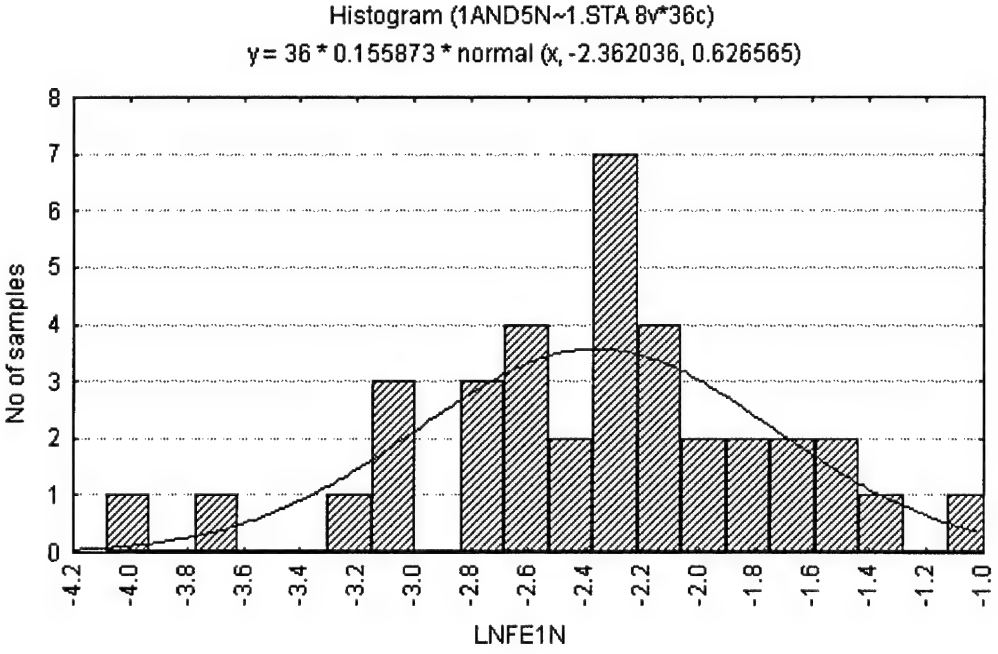

**Figure A3: January 1996 Samples, Normal Plot of Total Iron (5N HCl Extractable) Concentrations**

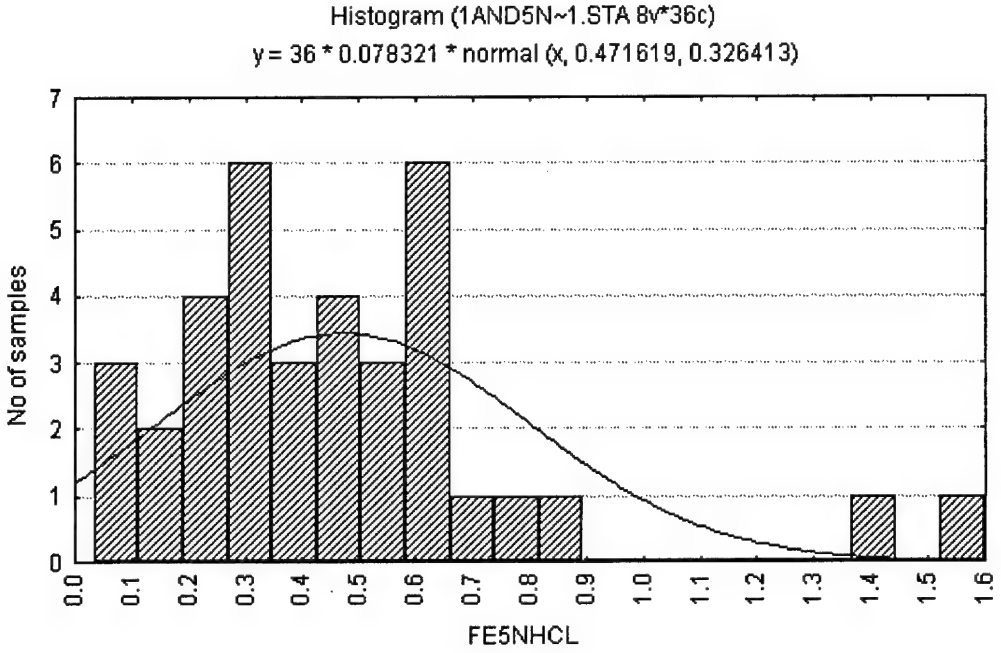


**Figure A4: January 1996 Samples, Normal Plot of Total Iron (5N HCl Extractable) Concentrations**

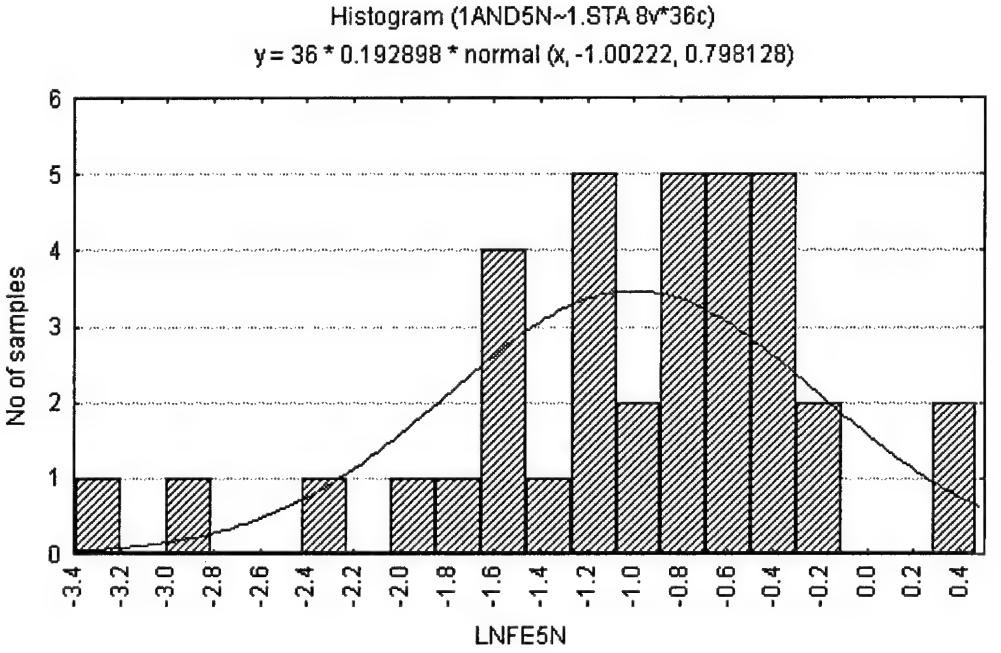

**Table A2: Amorphous Iron in NATS Core Samples**

(Results are the average of 2 analyses)

| | Top Level | | Middle Level | | Bottom Level | |
|---|---|---|---|---|---|---|
| Date and Location | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation |
| 1/96 A-1 | 0.140 | 0.032 | 0.110 | 0.006 | 0.150 | 0.004 |
| 9/96 A-1 | | | 0.160 | 0.015 | | |
| 3/97 A-1 | | | 0.181 | 0.002 | | |
| | | | | | | |
| 1/96 A-2 | 0.113 | 0.013 | 0.111 | 0.007 | 0.085 | 0.013 |
| 9/96 A-2 | | | 0.158 | 0.011 | | |
| 3/97 A-2 | | | 0.088 | 0.011 | | |
| | | | | | | |
| 1/96 A-3 | 0.225 | 0.005 | 0.065 | 0.002 | 0.039 | 0.009 |
| 9/96 A-3 | | | | | | |
| 3/97 A-3 | | | | | | |
| | | | | | | |
| 1/96 N-1 | 0.127 | 0.003 | 0.108 | 0.005 | 0.048 | 0.001 |
| 9/96 N-1 | | | | | | |
| 3/97 N-1 | | | 0.030 | 0.001 | | |
| | | | | | | |
| 1/96 N-2 | 0.111 | 0.000 | 0.108 | 0.001 | 0.380 | 0.000 |
| 9/96 N-2 | | | 0.060 | 0.001 | | |
| 3/97 N-2 | | | 0.044 | 0.001 | | |
| | | | | | | |
| 1/96 N-3 | 0.122 | 0.013 | 0.084 | 0.005 | 0.188 | 0.024 |
| 9/96 N-3 | | | 0.051 | 0.001 | | |
| 3/97 N-3 | | | 0.035 | 0.006 | | |
| | | | | | | |
| 9/96 N-4 | 0.088 | 0.009 | 0.074 | 0.000 | 0.112 | 0.000 |
| 3/97 N-4 | 0.065 | 0.004 | 0.125 | 0.003 | 0.021 | 0.006 |
| | | | | | | |
| 9/96 N-5 | 0.061 | 0.002 | 0.161 | 0.020 | 0.237 | 0.014 |
| 3/97 N-5 | 0.025 | 0.005 | 0.370 | 0.026 | 0.039 | 0.003 |

| | Top Level | | Middle Level | | Bottom Level | |
|---|---|---|---|---|---|---|
| Date and Location | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation |
| 1/96 M-1 | 0.100 | 0.005 | 0.090 | 0.005 | 0.080 | 0.013 |
| 9/96 M-1 | | | 0.170 | 0.001 | | |
| 3/97 M-1 | | | 0.052 | 0.006 | | |
| | | | | | | |
| 1/96 M-2 | 0.077 | 0.002 | 0.078 | 0.001 | 0.056 | 0.003 |
| 9/96 M-2 | | | 0.052 | 0.002 | | |
| 3/97 M-2 | | | 0.132 | 0.006 | | |
| | | | | | | |
| 1/96 M-3 | 0.204 | 0.023 | 0.062 | 0.000 | 0.216 | 0.037 |
| 9/96 M-3 | | | | | | |
| 3/97 M-3 | | | 0.033 | 0.018 | | |
| | | | | | | |
| 1/96 F-1 | 0.140 | 0.007 | 0.241 | 0.007 | 0.028 | 0.003 |
| 9/96 F-1 | | | 0.048 | 0.002 | | |
| 3/97 F-1 | | | 0.021 | 0.006 | | |
| | | | | | | |
| 1/96 F-2 | 0.157 | 0.004 | 0.031 | 0.008 | 0.099 | 0.003 |
| 9/96 F-2 | | | 0.106 | 0.002 | | |
| 3/97 F-2 | | | 0.045 | 0.011 | | |
| | | | | | | |
| 1/96 F-3 | 0.017 | 0.000 | 0.041 | 0.004 | 0.087 | 0.006 |
| 9/96 F-3 | | | | | | |
| 3/97 F-3 | | | | | | |

**Table A3. Fe (II) in NATS Core Samples**

| | Top Level | | Middle Level | | Bottom Level | |
|---|---|---|---|---|---|---|
| Date and Location | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation |
| 1/96 A-1 | 0.009 | 0.003 | 0.008 | 0.001 | 0.006 | 0.002 |
| 9/96 A-1 | | | 0.013 | 0.000 | | |
| 3/97 A-1 | | | 0.013 | 0.001 | | |
| 9/97 A-1 | | | 0.009 | 0.000 | | |
| | | | | | | |
| 1/96 A-2 | 0.007 | 0.003 | 0.011 | 0.002 | 0.009 | 0.000 |
| 9/96 A-2 | | | 0.014 | 0.003 | | |
| 3/97 A-2 | | | 0.007 | 0.001 | | |
| 9/97 A-2 | | | 0.005 | 0.001 | | |
| | | | | | | |
| 1/96 A-3 | 0.015 | 0.000 | 0.007 | 0.001 | 0.006 | 0.001 |
| 9/96 A-3 | | | | | | |
| 3/97 A-3 | | | | | | |
| 9/97 A-3 | | | 0.004 | 0.000 | | |
| | | | | | | |
| 1/96 N-1 | 0.007 | 0.001 | 0.009 | 0.001 | 0.002 | 0.000 |
| 9/96 N-1 | | | | | | |
| 3/97 N-1 | | | 0.001 | 0.000 | | |
| 9/97 N-1 | | | | | | |
| | | | | | | |
| 1/96 N-2 | 0.004 | 0.000 | 0.010 | 0.001 | 0.012 | 0.002 |
| 9/96 N-2 | | | 0.007 | 0.000 | | |
| 3/97 N-2 | | | 0.014 | 0.005 | | |
| 9/97 N-2 | | | 0.004 | 0.001 | | |
| | | | | | | |
| 1/96 N-3 | 0.008 | 0.000 | 0.015 | 0.002 | 0.013 | 0.002 |
| 9/96 N-3 | | | 0.007 | 0.001 | | |
| 3/97 N-3 | | | 0.004 | 0.001 | | |
| 9/97 N-3 | | | 0.003 | 0.001 | | |
| Sample | Top Level | | Middle Level | | Bottom Level | |
| 9/96 N-4 | 0.006 | 0.000 | 0.010 | 0.001 | 0.010 | 0.000 |
| 3/97 N-4 | 0.008 | 0.000 | 0.013 | 0.001 | 0.001 | 0.000 |
| 9/97 N-4 | 0.009 | | 0.003 | 0.000 | 0.012 | |
| | | | | | | |
| 9/96 N-5 | 0.010 | 0.001 | 0.028 | 0.005 | 0.010 | 0.001 |
| 3/97 N-5 | 0.001 | 0.000 | 0.021 | 0.001 | 0.008 | 0.001 |
| 9/97 N-5 | 0.007 | 0.001 | 0.015 | 0.001 | 0.008 | 0.000 |
| | | | | | | |

| | Top Level | | Middle Level | | Bottom Level | |
|---|---|---|---|---|---|---|
| Date and Location | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation |
| 1/96 M-1 | 0.005 | 0.001 | 0.008 | 0.001 | 0.010 | 0.0012 |
| 9/96 M-1 | | | 0.012 | 0.001 | | |
| 3/97 M-1 | | | 0.008 | 0.001 | | |
| 9/97 M-1 | | | 0.012 | 0.001 | | |
| | | | | | | |
| 1/96 M-2 | 0.009 | 0.001 | 0.007 | 0.001 | 0.003 | 0.001 |
| 9/96 M-2 | | | 0.011 | 0.001 | | |
| 3/97 M-2 | | | 0.011 | 0.001 | | |
| 9/97 M-2 | | | 0.008 | 0.000 | | |
| | | | | | | |
| 1/96 M-3 | 0.006 | 0.000 | 0.004 | 0.001 | 0.021 | 0.008 |
| 9/96 M-3 | | | | | | |
| 3/97 M-3 | | | 0.003 | 0.002 | | |
| 9/97 M-3 | | | | | | |
| | | | | | | |
| 1/96 F-1 | 0.009 | 0.002 | 0.019 | 0.000 | 0.002 | 0.0002 |
| 9/96 F-1 | | | 0.003 | 0.000 | | |
| 3/97 F-1 | | | 0.001 | 0.001 | | |
| 9/97 F-1 | | | | | | |
| | | | | | | |
| 1/96 F-2 | 0.019 | 0.002 | 0.002 | 0.001 | 0.012 | 0.0007 |
| 9/96 F-2 | | | 0.007 | 0.001 | | |
| 3/97 F-2 | | | 0.003 | 0.000 | | |
| 9/97 F-2 | | | 0.002 | 0.000 | | |
| | | | | | | |
| 1/96 F-3 | 0.001 | 0.000 | 0.008 | 0.001 | 0.012 | 0.0008 |
| 9/96 F-3 | | | | | | |
| 3/97 F-3 | | | | | | |
| 9/97 F-3 | | | | | | |
| | | | | | | |

**Table A4: Total Iron Content of the Field Samples as determined by 5N HCl extraction**

| | Top Level | | Middle Level | | Bottom Level | |
|---|---|---|---|---|---|---|
| Date and Location | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation |
| 1/96 A-1 | 0.59 | 0.077 | 0.77 | 0.04 | 0.33 | 0.018 |
| 9/96 A-1 | | | 1.11 | 0.01 | | |
| 3/97 A-1 | | | 1.14 | 0.55 | | |
| 9/97 A-1 | | | 0.96 | 0.06 | | |
| | | | | | | |
| 1/96 A-2 | 0.42 | 0.01 | 0.58 | 0.04 | 0.45 | 0.031 |
| 9/96 A-2 | | | 1.41 | 0.09 | | |
| 3/97 A-2 | | | 0.60 | 0.08 | | |
| 9/97 A-2 | | | 0.18 | 0.03 | | |
| | | | | | | |
| 1/96 A-3 | 0.70 | 0.04 | 0.23 | 0.07 | 0.14 | 0.004 |
| 9/96 A-3 | | | | | | |
| 3/97 A-3 | | | | | | |
| 9/97 A-3 | | | 0.33 | 0.02 | | |
| | | | | | | |
| 1/96 N-1 | 0.47 | 0.04 | 0.63 | 0.01 | 0.20 | 0.017 |
| 9/96 N-1 | | | 0.39 | 0.02 | | |
| 3/97 N-1 | | | | | | |
| 9/97 N-1 | | | | | | |
| | | | | | | |
| 1/96 N-2 | 0.38 | 0.02 | 0.54 | 0.00 | 0.78 | 0.049 |
| 9/96 N-2 | | | 0.47 | 0.02 | | |
| 3/97 N-2 | | | 0.34 | 0.21 | | |
| 9/97 N-2 | | | 0.30 | 0.01 | | |
| | | | | | | |
| 1/96 N-3 | 1.60 | 0.15 | 0.28 | 0.02 | 0.65 | 0.008 |
| 9/96 N-3 | | | 0.32 | 0.01 | | |
| 3/97 N-3 | | | 0.85 | 0.59 | | |
| 9/97 N-3 | | | 0.23 | 0.05 | | |
| Sample | Top Level | | Middle Level | | Bottom Level | |
| 9/96 N-4 | 0.54 | 0.03 | 0.85 | 0.43 | 0.27 | 0.01 |
| 3/97 N-4 | 0.71 | 0.07 | 0.81 | 0.00 | 0.20 | 0.007 |
| 9/97 N-4 | 0.31 | 0.04 | 0.29 | 0.01 | 0.49 | 0.043 |
| | | | | | | |
| 9/96 N-5 | 0.44 | 0.00 | 0.45 | 0.01 | 1.11 | 0.009 |
| 3/97 N-5 | 0.26 | 0.03 | 1.66 | 0.08 | 0.35 | 0.045 |
| 9/97 N-5 | 0.46 | 0.07 | 0.50 | 0.02 | 0.32 | 0.017 |

| | Top Level | | Middle Level | | Bottom Level | |
|---|---|---|---|---|---|---|
| Date and Location | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation | % Fe (w/w) | Standard Mean Deviation |
| 1/96 M-1 | 0.29 | 0.01 | 0.31 | 0.01 | 0.31 | 0.05 |
| 9/96 M-1 | | | 0.60 | 0.01 | | |
| 3/97 M-1 | | | 0.38 | 0.03 | | |
| 9/97 M-1 | | | 0.29 | 0.15 | | |
| | | | | | | |
| 1/96 M-2 | 0.23 | 0.00 | 0.46 | 0.23 | 0.38 | 0.019 |
| 9/96 M-2 | | | 0.29 | 0.02 | | |
| 3/97 M-2 | | | 0.88 | 0.05 | | |
| 9/97 M-2 | | | 0.32 | 0.04 | | |
| | | | | | | |
| 1/96 M-3 | 0.63 | 0.01 | 0.18 | 0.09 | 0.63 | 0.05 |
| 9/96 M-3 | | | | | | |
| 3/97 M-3 | | | 0.276 | 0.086 | | |
| 9/97 M-3 | | | | | | |
| | | | | | | |
| 1/96 F-1 | 0.51 | 0.02 | 0.89 | 0.05 | 0.05 | 0.004 |
| 9/96 F-1 | | | 0.46 | 0.00 | | |
| 3/97 F-1 | | | | | | |
| 9/97 F-1 | | | | | | |
| | | | | | | |
| 1/96 F-2 | 0.60 | 0.01 | 0.09 | 0.00 | 0.50 | 0.009 |
| 9/96 F-2 | | | 0.25 | 0.02 | | |
| 3/97 F-2 | | | | | | |
| 9/97 F-2 | | | 0.09 | 0.05 | | |
| | | | | | | |
| 1/96 F-3 | 0.03 | 0.00 | 0.20 | 0.02 | 0.32 | 0.007 |
| 9/96 F-3 | | | | | | |
| 3/97 F-3 | | | | | | |

**Table A5: Fe/Si ratios of field aquifer sediment samples as determined by SEM/EDS**

| Date and Location | Top | Middle | Bottom | Date and Location | Top | Middle | Bottom |
|---|---|---|---|---|---|---|---|
| 1/96 A-1 | | 1.38 | | 1/96 M-1 | | | |
| 9/96 A-1 | | | | 9/96 M-1 | | 2.31 | |
| 3/97 A-1 | | 3.23 | | 3/97 M-1 | | 1.68 | |
| 9/97 A-1 | | 3.48 | | 9/97 M-1 | | | |
| | | | | | | | |
| 1/96 A-2 | | 1.54 | | 1/96 M-2 | | 0.97 | |
| 9/96 A-2 | | 2.11 | | 9/96 M-2 | | 1.51 | |
| 3/97 A-2 | | 2.49 | | 3/97 M-2 | | 1.88 | |
| 9/97 A-2 | | 0.77 | | 9/97 M-2 | | 1.27 | |
| | | | | | | | |
| 1/96 A-3 | | 1.65 | | 1/96 M-3 | | 0.88 | |
| 9/96 A-3 | | | | 9/96 M-3 | | | |
| 3/97 A-3 | | | | 3/97 M-3 | | | |
| 9/97 A-3 | | | | 9/97 M-3 | | | |
| | | | | | | | |
| 1/96 N-1 | | 1.22 | | 1/96 F-1 | | | |
| 9/96 N-1 | | | | 9/96 F-1 | | | |
| 3/97 N-1 | | | | 3/97 F-1 | | | |
| 9/97 N-1 | | | | 9/97 F-1 | | | |
| | | | | | | | |
| 1/96 N-2 | | 1.31 | | 1/96 F-2 | | 0.92 | |
| 9/96 N-2 | | | | 9/96 F-2 | | 1.48 | |
| 3/97 N-2 | | | | 3/97 F-2 | | 1.30 | |
| 9/97 N-2 | | 0.92 | | 9/97 F-2 | | 0.34 | |
| | | | | | | | |
| 1/96 N-3 | | 1.47 | | 1/96 F-3 | | | |
| 9/96 N-3 | | 1.92 | | 9/96 F-3 | | | |
| 3/97 N-3 | | 1.85 | | 3/97 F-3 | | | |
| 9/97 N-3 | | 1.14 | | 9/97 F-3 | | | |
| | | | | | | | |
| 9/96 N-4 | 2.85 | 2.90 | 1.52 | | | | |
| 3/97 N-4 | 4.13 | 4.82 | 1.50 | | | | |
| 9/97 N-4 | 0.87 | 1.01 | 1.07 | | | | |
| | | | | | | | |
| 9/96 N-5 | 1.38 | 1.56 | 2.67 | | | | |
| 3/97 N-5 | 4.34 | 8.20 | 1.77 | | | | |
| 9/97 N-5 | 2.60 | 1.70 | 0.79 | | | | |

**Table A6: Fe (II) Concentration (mg/L) in Vials after 1 and 5 Months**

(Detection Limit = 0.05pppm)

| Sample Name | 1 Month Fe (II) Concentration (ppm) | 5 Month Fe (II) Concentration (ppm) |
|---|---|---|
| CONTROL A1 | 0.49 | 0.22 |
| CONTROL A2 | <0.05 | 0.26 |
| CONTROL B1 | 119.51 | 16.48 |
| CONTROL B2 | 128.26 | 16.25 |
| 50 A1 | 5.30 | 3.27 |
| 50 A2 | 4.73 | 2.53 |
| 50 A3 | 7.27 | 4.91 |
| 50 B1 | 247.68 | |
| 50 B2 | 160.63 | |
| 100 A1 | 21.22 | 6.51 |
| 100 A2 | 18.86 | |
| 100 A3 | 19.74 | |
| 100 B1 | 210.50 | 15.64 |
| 100 B2 | 98.08 | 14.29 |
| 100 B3 | 108.14 | 15.58 |
| 200 A1 | 40.44 | 8.02 |
| 200 A2 | 42.45 | 15.06 |
| 200 A3 | 40.35 | 17.17 |
| 200 B2 | 200.00 | 16.00 |
| 200 B3 | 158.88 | 15.06 |
| 400 A1 | 89.76 | 36.59 |
| 400 A2 | 130.01 | |
| 400 A3 | 112.95 | |
| 400 B1 | 368.85 | 15.73 |
| 400 B2 | 265.18 | 10.79 |
| 400 B3 | 296.24 | 16.85 |
| 400 B3 | 296.68 | |
| 800 A1 | 765.70 | 87.68 |
| 800 A2 | 560.98 | |
| 800 B1 | 682.59 | 90.24 |
| 800 B2 | 753.46 | 87.12 |
| 800 B3 | 783.20 | 96.40 |

**Table A7: Fe(II) Concentration (ppm) in the Batch Microbial Experiment**

| Naturally Occurring | | Triplicate 1 | Triplicate 2 | Triplicate 3 | Average Fe (ppm) | %CV |
|---|---|---|---|---|---|---|
| Acetate Vials | Seds. Only | 3.49 | 4.70 | 3.56 | 3.92 | 17.35 |
| | Seds.+ Microbes | 4.07 | 3.68 | 3.65 | 3.80 | 6.15 |
| | Microbes Only | 0.44 | 0.32 | 0.39 | 0.38 | 15.21 |
| | Media Only | 0.03 | 0.04 | | <0.05 | |
| Toluene Vials | | | | | | |
| | Seds. Only | 4.15 | 3.62 | 4.70 | 4.16 | 13.02 |
| | Seds.+ Microbes | 3.77 | 3.16 | 5.70 | 4.21 | 31.48 |
| | Microbes Only | 0.32 | 0.42 | 0.44 | 0.40 | 16.21 |
| | Media Only | 0.04 | 0.04 | | <0.05 | |
| | | | | | | |
| GS-15 Innoculates | | | | | | |
| Acetate Vials | Seds. Only | 4.72 | 2.08 | 3.83 | 3.54 | 37.96 |
| | Seds.+ Microbes | 4.65 | 3.67 | 0.47 | 2.93 | 74.63 |
| | Microbes Only | 2.62 | 2.12 | 2.44 | 2.39 | 10.64 |
| | Media Only | 0.03 | 0.03 | | 0.03 | 5.04 |
| | | | | | | |
| | Abiotic Control | <0.1 | <0.1 | | <0.05 | |